THE EFFECT OF ANTISERUM AGAINST THE SOLUBLE TOXIC SUBSTANCE OF BACILLUS COLI IN BACILLUS COLI PERITONITIS.

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It has been long suspected that toxins play an important part in peritonitis. Clinical and experimental observers have noticed signs and symptoms believed to be due to absorption of toxic material. This toxic state is said to be present before the evident onset of peritonitis and also during the course of the disease. However, no proof of actual absorption of any toxic substance from the peritoneal cavity in peritonitis has been demonstrated so far.

Wegner (1), 1876, injected bacteria into the peritoneal cavity of animals and found that death occurred within a few hours and before any signs of peritonitis became apparent. Pawlowsky (2), 1887, and others repeated Wegner's experiments with similar results. In the following two decades this phase of peritonitis received no additional important contribution.

Smith and TenBroeck (3), 1915, demonstrated a soluble toxic substance of fowl typhoid bacillus and Ecker (4), 1917, showed a similar substance in the cultures of the paratyphoid bacillus. Antisera against some members of the colon-typhoid group (paratyphoid, enteritidis, *suipestifer*) were demonstrated by Ecker (4), Branham (5), and Ecker and Richardson (6).

A soluble toxic substance from young cultures of *B. coli* was demonstrated by Aronovitch (7), 1920, Zinsser (8), 1920, Branham (5), 1925. Evidence supplied by these workers makes it appear that the toxic substance is not an endotoxin or a split-product of the culture medium but belongs rather to the group of exotoxins. Zinsser (8) expressed doubt as to the specificity of this toxic substance elaborated by the colon bacillus. None of these workers succeeded in producing an antiserum against the soluble toxic substance of *B. coli*.

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Injection of a Centrifugalized and Heated Culture of B. coli into the Peritoneal Cavity of Rabbits.

18 to 24 hour cultures of colon bacillus were injected into the peritoneal cavity of rabbits. The animals died in from 4 to 5 hours and showed only a slight degree of hyperemia of the peritoneum. In order to determine whether the soluble toxic substance produced by the

TABLE I.

Intraperitoneal Injection of 30 Cc. of Centrifugalized and Heated B. coli Culture.

No. of rabbit.	Weight.	Time of death after injection.	Symptoms.
	gm.	*hrs.*	
1	2000	$8\frac{1}{4}$	In 1–$1\frac{1}{2}$ hrs. weakness of hind legs, increased respiration. In 2–3 hrs. prostration. In 6–7 hrs. the animal is lying on his side breathing stertorously. Death follows.
2	1720	$8\frac{1}{2}$	" "
3	1920	$8\frac{1}{2}$	" "
4	1990	9	" "
5	1450	9	" "
6	1490	$10\frac{1}{2}$	" "

TABLE II.

Intraperitoneal Injection of 30 Cc. of Sterile Broth.

No. of rabbit.	Weight.	Death or survival.	Symptoms.
	gm.		
7	1820	Survived.	None.
8	1700	"	"
9	1540	"	"
10	1770	"	"

young cultures of *B. coli* was responsible for the death of the animals, the toxin-bearing part of the culture was separated.

B. coli (Culture 300) isolated from the blood of a patient was grown on a 0.3 per cent Liebig meat extract broth (1 per cent Difco peptone; 0.5 per cent NaCl; pH 7.4) for 18 to 24 hours. The broth culture was then centrifugalized at high speed for 2 hours. The supernatant fluid was poured off and heated for $\frac{1}{2}$ hour at 60°C. Subsequent cultures of the supernatant fluid were sterile. Each of six

rabbits was injected intraperitoneally with 30 cc. of the centrifugalized and heated broth culture. After an incubation period varying from 1 to 1½ hours the animals showed weakness; in 2 to 3 hours prostration; between the 6th and 7th hours they were lying on the side and unable to rise and had stertorous respiration. Death ensued in 8¼ to 10½ hours. The necropsy revealed only hyperemia of the visceral and parietal peritoneum and a little red-tinged cloudy free fluid.

Some of the preliminary experiments showed that the toxic substance is produced as early as the 6th hour of bacterial growth.

TABLE III.

Production of an Antiserum by Weekly Injections.

No. of rabbit.	Weight.	No. of injections.	Outcome.	Remarks.
	gm.			
11	1740	6	Killed.	Lost 36 gm.; very thin.
12	1920	6	"	" 45 " " "
13	1800	12	"	" 46 " hind legs paralyzed.
14	2170	2	Died.	Pneumonia with empyema.
15	1570	10	Killed.	Lost 21 gm.; very thin.
16	1680	7	Died.	Necropsy did not reveal the cause of death.

TABLE IV.

Production of an Antiserum by Biweekly Injections.

No. of rabbit.	Weight.	No. of injections.	Outcome.	Remarks.
	gm.			
17	2120	10	Killed.	Lost 39 gm.; very thin and weak.
18	1980	7	"	Paralysis of hind legs; weakness; paralysis of anal sphincter.
19	1920	5	"	Difficult breathing; weakness.
20	2650	10	"	Lost 21 gm.; slight weakness.
21	3500	10	"	" 32 "
22	2270	10	"	" 17 "

Four rabbits were injected intraperitoneally with 30 cc. of a sterile meat extract broth. The animals showed no demonstrable symptoms and all survived.

Production of an Antiserum against the Soluble Toxic Substance of B. coli.

B. coli (Culture 300) was grown for 18 to 24 hours in a meat extract broth, centrifugalized for 2 hours, and the supernatant fluid immediately injected into

the marginal vein of a rabbit. Twelve rabbits were divided into two groups, six animals in each group. One group was given weekly intravenous injections, the other biweekly. The initial dose varied from 0.5 to 1.0 cc. with a weekly increase of 1.0 cc. up to 10.0 so that at the tenth injection some were getting 9.5 cc. and others 10.0 cc. The maximum total volume that an animal received aggregated 75 cc. in 12 weeks.

Out of twelve animals two died; one had bronchopneumonia and in the other no anatomical lesions were found. The surviving animals showed a slight loss in weight and a perceptible weakness; two rabbits had a paralysis of the hind extremities.

The animals were bled aseptically and the serum tested against a known lethal dose for white rats of an 18 hour whole broth culture of *B. coli*. It was found that 5 cc. of the 18 hour broth culture of *B. coli* (No. 300) killed a white rat in 8 hours. 3 cc. of the antiserum were incubated for 1 hour with 5 cc. of the *B. coli* broth culture and injected into the peritoneal cavity of a white rat. The animal survived. The serum of the animal which received six injections and the one which received twelve injections were tested but no difference in potency could be discovered. Tested in a rabbit it was found that 10 cc. of the antiserum injected intravenously were required to protect against 30 cc. of an 18 hour broth culture of *B. coli* given intraperitoneally.

The agglutinating titer of the antiserum against *B. coli* was determined to be 1:50.

Injection of Antiserum in Rabbits with B. coli Peritonitis.

Nine rabbits varying in weight from 1740 to 2270 gm. received each an intraperitoneal injection of 25 cc. of an 18 hour broth culture of *B. coli* (No. 300). The animals evinced no signs of illness for 1 to 1½ hours. At the end of that time they stopped eating, became weak, and showed an increased respiratory rate. Those animals that died in 2 hours rapidly became weaker and prostrated, dying with a few twitchings and generalized spasms. The peritoneum was hyperemic and the cavity contained some free cloudy yellow fluid that gave an abundant growth of *B. coli*. The animals that did not succumb for 2 and 3 hours longer presented a similar set of symptoms. There was a more marked inflammation in the peritoneal cavity of the latter animals. A culture from the peritoneal fluid gave an abundant growth of *B. coli*.

The dose of the *B. coli* culture given was five times the usual lethal dose for a rabbit. It was found that 5 cc. killed in 12 hours a rabbit weighing 1900 gm. However, owing to the individual variation of the animals it was deemed advisable to multiply the dose at least five times to insure a lethal effect.

Nine rabbits varying in weight from 1720 to 2520 gm. were each given intraperitoneal injection of 25 cc. of the *B. coli* broth culture (from the same bacterial

suspension that was used in the nine rabbits in Table V). Immediately after the intraperitoneal injection each animal received intravenously 10 cc. of the antiserum.

Another group of three rabbits was given similar intraperitoneal doses of the *B. coli* broth culture but the intravenous injection of the antiserum was withheld for ½ hour to allow for a considerable absorption of the toxic substance.

TABLE V.

Intraperitoneal Injection of 25 Cc. of an 18 Hour B. coli Broth Culture.

No. of rabbit.	Weight.	Outcome.	Remarks.
	gm.		
23	2570	Died in 1¾ hrs.	All rabbits showed an incubation period of 1–1½ hrs. at the end of which the respiration became increased, there was apparent weakness, the animals lay flaccid, death coming on with a few spasms. The intestines in two-thirds of the animals were ballooned out.
24	1740	" " 1¾ "	
25	2270	" " 1¾ "	
26	1980	" " 2 "	
27	1950	" " 2 "	
28	1920	" " 4 "	
29	2140	" " 3¾ "	
30	2120	" " 1½ "	
31	2020	" " 1½ "	

TABLE VI.

Intraperitoneal Injection of 25 Cc. of B. coli Broth Culture and Intravenous Injection of the Antiserum.

No. of rabbit.	Weight.	Outcome.	Remarks.
	gm.		
32	2520	Survived.	The animals in 1–1½ hrs. after injection were listless, did not eat, but otherwise showed no ill effect. In 6 hrs. they were apparently well. The two rabbits that died did not present the symptoms observed in animals in Table V.
33	2100	"	
34	2250	"	
35	2020	"	
36	1720	Died in 8 hrs.	
37	2190	Survived.	
38	1800	Died in 8–10 hrs.	
39	1980	Survived.	
40	2000	"	

In the first group of nine rabbits seven survived; in the second group all survived. In 1 to 1½ hours the animals that survived in both groups became somewhat listless and stopped eating but 6 hours later showed no further signs of discomfort.

Two animals in the first group died in 8 to 10 hours after the intraperitoneal

injection. However, they did not show the marked weakness with prostration that was observed in the rabbits of Table V. The peritoneal cavity of these two rabbits showed a mild inflammation and a culture from the peritoneal fluid gave an abundant growth of *B. coli.*

TABLE VII.

Intraperitoneal Injection of 25 Cc. of an 18 Hour B. coli Broth Culture and Intravenous Injection of Antiserum ½ Hour Later.

No. of rabbit.	Weight.	Outcome.	Remarks.
	gm.		
41	2020	Survived.	The animals showed the same symptoms as those in Table VI.
42	1950	"	
43	1850	"	

TABLE VIII.

Intraperitoneal Injection of 25 Cc. of B. coli Broth Culture and Intravenous Injection of 10 Cc. of Normal Serum.

No. of rabbit.	Weight.	Outcome.	Remarks.
	gm.		
44	1750	Died in 2 hrs.	The animals showed the same symptoms as those in Table V.
45	2190	" " 1¾ "	
46	2050	" " 2¼ "	
47	1800	" " 4½ "	
48	1960	" " 4½ "	

TABLE IX.

Intraperitoneal Injection of 25 Cc. of B. coli Broth Culture and Intravenous Injection of 10 Cc. of Agglutinating Serum.

No. of rabbit.	Weight.	Outcome.	Remarks.
	gm.		
49	1650	Died in 3¼ hrs.	The animals showed the same symptoms as those in Table V.
50	2240	" " 3 "	
51	1900	" " 6 "	
52	1900	" " 6 "	

Five rabbits were each given an intraperitoneal injection of 25 cc. of the *B. coli* broth culture. Immediately after, each animal received intravenously 10 cc. of normal rabbit serum. All the animals succumbed in from 1¾ to 4½ hours with the same trend of symptoms as described for the group in Table V.

An agglutinating serum against washed *B. coli* was produced with an agglutinating titer of 1:1800. Four rabbits were given each intraperitoneally 25 cc. of the *B. coli* broth culture and immediately after 10 cc. of the *B. coli* agglutinating serum intravenously. The four rabbits succumbed in 3 to 6 hours after the intraperitoneal injection with a similar trend of symptoms as described for the group in Table V. Cultures of the peritoneal fluid gave an abundant growth of *B. coli.*

Production of a Serofibrinous Peritonitis in Rabbits by Intraperitoneal Injection of 6 Hour Cultures of B. coli.

When rabbits were injected intraperitoneally with 18 to 24 hour cultures of *B. coli,* the peritoneum revealed only a hyperemia. Three rabbits were given intraperitoneally 30 cc. of a 6 hour culture of *B. coli.* Two animals died 24 hours later, the third one survived. The rabbits that succumbed showed grossly a fibrinous exudate on the visceral and parietal peritoneum and diaphragm with a little cloudy free yellow fluid in the peritoneal cavity. A microscopic examination of the peritoneum revealed a moderate amount of fibrin on the serous surface, an inflammatory edema, and an exudation of polymorphonuclear leucocytes.

The rabbit that survived was sacrificed 48 hours after the intraperitoneal injection. The peritoneal cavity of that animal revealed nothing abnormal. Repeated similar experiments demonstrated that 30 per cent of rabbits injected with 6 hour broth cultures of *B. coli* do not show any peritonitis. Apparently, the individual resistance of the animal is an important factor.

SUMMARY.

When colon bacilli in sufficient numbers were injected into the peritoneal cavity of rabbits the animals died within a few hours. There was an incubation period of 1 to 1½ hours which was followed by a set of symptoms that culminated in death. The peritoneum, however, showed only very slight changes.

It was thought that the soluble toxic substance of *B. coli* which was demonstrated by Aronovitch, Zinsser, and Branham may be an important lethal factor. Young cultures of *B. coli* in broth were centrifugalized and after killing the remaining bacteria by heat the supernatant fluid was injected into the peritoneal cavity of rabbits. There ensued the same set of symptoms terminating in death which were seen on the injection of the whole culture when the supernatant fluid alone was given.

The potency of the toxic substance is variable. This variability is probably in part inherent in the material and in part depends upon the individual resistance of the animals.

Rabbits were injected intravenously with the supernatant fluid of centrifugalized young cultures of *B. coli* and an antiserum was elaborated against the soluble toxic substance of this organism.

When this antiserum was given intravenously to rabbits immediately or ½ hour after receiving intraperitoneally five times the usual lethal dose of *B. coli*, ten out of twelve animals survived.

CONCLUSIONS.

1. From young cultures of *B. coli* (18 to 24 hours) a soluble toxic substance was obtained and an antiserum against this toxic substance was produced.
2. The soluble toxic substance is the principal lethal factor when cultures of *B. coli* are injected into the peritoneal cavity of rabbits.
3. Intravenous administration of the antiserum can avert death in rabbits previously injected intraperitoneally with lethal doses of *B. coli*.

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THE REACTION OF COW'S MILK TO BLOOD SERUM PRECIPITIN.

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Osborne and Wakeman[1] isolated in a high degree of purity four proteins from cow's milk: casein, lactalbumin, lactoglobulin, and an alcohol-soluble protein. By chemical methods Crowther and Raistrick[2] had previously been unable to show that the lactoglobulin differed from serum globulin. Schlossmann and Moro[3] had called attention to the fact that a precipitin prepared by injecting animals with milk whey reacts with blood serum. Wells and Osborne[4] using the anaphylactic phenomenon showed that the four proteins obtained by Osborne and Wakeman were antigenically distinct. They further called attention to the fact that lactoglobulin sensitized guinea pigs to beef serum, and that test animals sensitized to beef serum reacted when subsequently injected with lactoglobulin. The inference then is that serum globulin and lactoglobulin are the same, since they cannot be differentiated by chemical or serologic methods. Since it has been well established that globulin is a normal constituent of milk, it should be possible to recognize its presence in milk when tested with a suitable serum precipitin. Furthermore, if the reaction of normal milk to a specific serum precipitin is once established, departures from the normal can readily be detected.

EXPERIMENTAL.

Rabbits were immunized with cow serum until a high titered precipitin had been obtained. The sera were mixed and stored in the refrigerator. Throughout,

[1] Osborne, T. B., and Wakeman, A. J., *J. Biol. Chem.*, 1918, xxxiii, 7.

[2] Crowther, C., and Raistrick, H., *Biochem. J.*, 1916, x, 434.

[3] Schlossmann, A., and Moro, E., *Münch. med. Woch.*, 1903, l, 597.

[4] Wells, H. G., and Osborne, T. B., *J. Infect. Dis.*, 1921, xxix, 200.

TABLE I.

The Reaction of Cow's Milk to Serum Precipitin.

Cow No.	Dilutions of milk tested with 0.1 cc. of serum precipitin.						Number and kind of bacteria per 1 cc. of milk.
	1:20	1:40	1:80	1:160	1:320	1:640	
1	+++*	++	++	+	−	−	540. No streptococci.
2	+++	++	++	+	+	−	7,500. 90 per cent micrococci and 5 per cent hemolytic streptococci.
3	+++	++	++	+	−	−	70. No streptococci.
4	+++	+++	++	+	−	−	600. 40 per cent non-hemolytic streptococci.
5	++	++	+	−	−	−	1,100. No streptococci.
6	+++	++	+	+	−	−	900. 15 per cent hemolytic streptococci.
7	+++	+++	++	++	+	−	19,200. 85 per cent hemolytic streptococci.
8	+++	++	+	±	−	−	800. No streptococci.
9	+++	+++	++	+	−	−	2,800. 20 per cent hemolytic streptococci.
10	+++	+	+	±	−	−	75. No streptococci.
11	+++	++	+	+	−	−	680. Micrococci.
12	+++	+++	++	+	+	±	2,000. 85 per cent hemolytic streptococci.
13	+++	++	+	±	−	−	810. No streptococci.
14	+++	++	+	+	±	−	5,600. 70 per cent hemolytic streptococci.
15	+++	+++	++	++	+	±	1,060. 10 per cent hemolytic streptococci.
16	+++	++	+	+	−	−	2,800. 80 per cent non-hemolytic streptococci.
17	+++	+++	++	+	+	−	1,600. No streptococci.
18	+++	++	++	+	±	−	940. 60 per cent non-hemolytic, and 5 per cent hemolytic streptococci.
19	+++	++	++	+	±	−	1,450. A few non-hemolytic streptococci.
20	+++	++	+	±	−	−	100. No streptococci.

* The amount of precipitation is recorded in the following manner: +++, the maximum, a heavy precipitate; ++, a more moderate accumulation at the bottom of the tube; +, a slight but well defined precipitate; ±, a trace of sediment which fails to appreciably cloud the tube on agitation.

a precipitin was used which in amounts of 0.1 cc. would detect $\frac{1}{40,960}$ cc. of cow serum.

The milk was largely freed of fat by permitting it to stand in the refrigerator overnight. The tests were made by diluting the milk with NaCl solution, and adding to each tube containing 1 cc. of the diluted milk 0.1 cc. of the specific serum. The tubes were incubated 2 hours and refrigerated overnight.

Experiment 1.—At various times milk from 20 cows was obtained directly from the milk pails. In addition to the tests for serum proteins, samples were plated in blood agar and films were examined for the presence of excessive numbers of leucocytes and bacteria. The results of the precipitin tests and plate cultures are recorded in Table I.

It can safely be said that normal milk when tested with amounts of specific blood serum precipitin which will detect $\frac{1}{40,960}$ cc. of cow serum, will react in dilutions of 1:80 and 1:160. Six of the samples

TABLE II.

The Reaction of Fore Milk and of Strippings When Tested with Serum Precipitin.

Cow No.		Dilutions of milk.				
		1:40	1:80	1:160	1:320	1:640
21	Fore milk.	++	+	+	−	−
21	Strippings.	++	+	+	−	−
22	Fore milk.	+++	++	+	+	−
22	Strippings.	+++	++	+	±	−

reacted in a dilution of 1:320, and two but slightly at 1:640. In the main, then, it appears that there is a definite level at which milk will react. Several of the cows harbored a considerable number of organisms in the milk. The reactions usually occurred at a higher dilution in such instances, although this was not always the case.

It seemed probable that the milk withdrawn from the udder at the end of milking might be richer in blood protein than that obtained at first. That such is not the case is indicated in Table II.

It is true that milk drawn from normal udders will react in rather definite dilutions to a definite amount of serum precipitin. The end-point varies within relatively narrow limits, but is not greatly influenced by the presence or absence of moderate numbers of streptococci or the other usual udder bacteria.

It is well known that during pregnancy when the udder is no longer secreting there accumulates a distinctly serous liquid. This rapidly increases in amount toward the end of pregnancy. With birth and the consequent frequent emptying of the udder the appearance of the secretion changes. The reaction of the colostrum and of the milk shortly after parturition is given in Table III.

TABLE III.

The Reaction of the Colostrum and of the Milk Shortly after Parturition to Serum Precipitin.

Cow No.		Dilutions of udder fluid or milk.								
		1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	1:20,480
1	Before calving.			+++	+++	+++	+++	+++	++	+
	Day of calving.			+++	+++	+++	++	+	+	±
	Days after calving.									
	1		+++	+++	++	+	+	±	−	−
	2		+++	++	++	+	±	−	−	−
	3		+++	++	+	±	−	−	−	−
	4	+++	+++	++	+	±	−	−	−	−
	6	+++	+++	++	+	±	−	−	−	−
	7	+++	++	+	+	±	−	−	−	−
2	Day of calving.		+++	+++	++	+	+	±	−	−
	Days after calving.									
	1		+++	+++	+++	++	+	±	−	−
	2		+++	+++	++	+	+	±	−	−
	3		+++	++	+	+	+	−	−	−
	4		+++	++	+	+	±	−	−	−
	5		++	+	+	+	−	−	−	−
	7		++	+	+	−	−	−	−	−
	9		+	+	+	−	−	−	−	−
	14	++	+	+	−	−	−	−	−	−

The results of the precipitin tests show that the udder fluid just before parturition is rich in serum proteins. Their concentration within the udder is temporary; at first they diminish rapidly as soon as the udder is regularly emptied, and then fall gradually to the normal level.

The presence of serum in the milk from inflamed udders has led several to suggest that its presence in excessive amounts may be used

for diagnostic purposes. Schern[5] has shown that rennet fails to coagulate milk from inflamed udders. This he attributes to the transudation of blood serum which is said to contain anti-rennet. Baker and Van Slyke[6] showed that the milk from diseased udders was on the whole more alkaline when tested with brom cresol purple than normal milk.

It seemed desirable to test the blood protein of several samples of milk drawn from inflamed udders. In Table IV the results of these tests and a general description of the character of the secretion are recorded.

From the observations recorded in Table IV it becomes apparent that even in milder cases of mastitis, such as that of Cow 1049, there is a considerable increase in the serum content of the milk from the affected quarter. As the process becomes more severe serum passes into the udder to a greater degree. The increase of serum is, however, confined only to the involved quarter, since the results of a number of tests of the milk from the uninvolved quarters have given reactions at only the dilutions which may be considered the normal level.

The ability of the precipitin test to readily detect abnormalities in the secretion has been shown. It must be borne in mind, however, that the cases of mastitis could also be detected by careful clinical examination. However, under usual herd conditions it is possible that milk from definitely involved quarters or from fresh cows may enter the herd supply. That such a test would be of considerable importance as an aid in detecting such undesirable additions is brought out in Table V. In this experiment samples of bottled milk were mixed with various amounts of exudate from a severely involved quarter. The various dilutions of the mastitis exudate in market milk were then tested with the serum precipitin.

It has been shown that the addition of even as little as one-eightieth part of mastitis milk to the bottled milk will increase the serum titer four- to eightfold. It might be said that the addition of the mastitis milk would so change the character of the herd milk that it would become readily apparent by methods in use; such was not the case.

[5] Schern, cited by Klein, L. A., Principles and practice of milk hygiene, Philadelphia and London, 1917.

[6] Baker, J. C., and Van Slyke, L. L., *New York State Agric. Exp. Station, Techn. Bull. 71*, 1919.

TABLE IV.

The Reaction of Milk Obtained from Cases of Mastitis to Serum Precipitin.

	Dilutions of milk from quarters of udder affected with mastitis.						
	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240
Cow 1049.—Mastitis of 2 days standing. Milk normal in color but contains tiny flocculi. Fails to coagulate on boiling. 5,500 non-hemolytic streptococci per cc.	+++	++	+	+	±	−	−
Cow G.A. 8.—Mastitis of 3 wks. standing. Atrophy of affected quarter. Milk normal except for tiny flocculi. 8,500 non-hemolytic streptococci per cc.	+++	++	+	+	−	−	−
Cow 4836.—Mastitis of 2 days standing. Milk yellow in color, thick in consistency, and contains yellow flocculi. 100 non-hemolytic streptococci per cc.	+++	++	+	+	+	+	±
Cow 1375.—Mastitis of 3 days standing. Milk tinged with pink and contains small flocculi. Fails to coagulate on boiling. 12,000 non-hemolytic streptococci per cc.	+++	+++	++	+	+	±	−
Cow 3321.—Within 24 hrs. of onset. Milk slightly watery, otherwise normal. Fails to coagulate on boiling. 200 non-hemolytic streptococci per cc.	+++	+++	++	+	+	±	±
Cow 4910.—Mastitis of 5 days standing. Recovering. Milk yellow and contains flocculi. 20 non-hemolytic streptococci per cc.	+++	+++	+++	+++	++	+	±

The reaction of the mastitis milk was definitely alkaline, since it became a deep purple on the addition of brom cresol purple. The bottled milk was distinctly acid when tested with the same indicator. The

addition of the mastitis exudate in the dilutions specified in the table failed to appreciably change the reaction of the milk.

It seemed probable that pasteurization of the milk might influence the precipitin test, provided the milk was heated sufficiently to coagulate the protein. To ascertain at what temperature the blood protein

TABLE V.

The Effect of Adding Mastitis Exudate on the Serum Content of Milk.

	Dilutions of milk.								
	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	1:20,480	1:40,960
Market milk	++	+	−	−	−	−	−	−	−
Mastitis exudate				+++	++	++	++	++	±
1 part of mastitis exudate diluted with 10 parts of market milk	+++	+++	+++	+	+	±	−	−	−
Diluted with 20 parts	+++	+++	++	+	±	±	−	−	−
" " 40 "	+++	++	++	+	±	−	−	−	−
" " 80 "	+++	++	+	+	±	−	−	−	−

TABLE VI.

The Effect of Heating Milk on the Precipitin Test.

	Dilutions of milk.				
	1:40	1:80	1:160	1:320	1:640
Raw milk	+++	++	+	±	−
Heated 20 min. at					
55–56°C.	+++	++	+	±	−
62–62.5°C.	+++	++	+	±	−
65–66°C.	+++	++	+	±	−
68–69°C.	−	−	−	−	−

in milk was no longer capable of reacting with the serum precipitin the following experiment was performed.

Mixed milk partially freed of fat was divided into lots and heated for 20 minutes in the water bath at various temperatures. Each lot was then tested with serum precipitin. The results are shown in Table VI.

It is clear that the reacting protein in milk is relatively resistant to heat, since milk heated at a temperature between 65° and 66°C. for 20 minutes still reacted as well as the raw sample. If, however, the temperature is increased to 68–69°C. for the same period, the milk ceases to react.

In addition to the observations cited a number of samples of milk obtained from cows harboring a considerable number of streptococci in the udder have been tested. The milk showed no gross abnormalities. Since the series is small only two cases will be cited. Cow 3954 had had several attacks of mastitis in the previous lactation period. 18 days after parturition plate cultures showed 20,000 non-hemolytic streptococci per cc. The milk reacted in a dilution of 1:1,280. In Cow 4784, blood had been observed on two occasions in the milk. 1 month after the last attack the milk contained 50,000 streptococci per cc. and gave a strong precipitin reaction in a dilution of 1:1,280. Similar results were obtained in two other instances.

DISCUSSION AND SUMMARY.

It seems well established that during normal lactation there passes a slight but well defined quantity of blood protein into the udder. When a specific serum precipitin is applied to diluted milk the reaction occurs at a relatively uniform level of dilution. This line may well be considered the normal level of blood protein elimination. In the main the serum protein level is the same when mastitis streptococci are present as when they are absent, provided they are not too numerous. On the other hand it has been shown that when streptococci are present in large numbers the milk while apparently normal reacts at dilutions considerably higher than the normal.

In the non-lactating udder, particularly during the latter end of gestation, serum proteins accumulate. Little and Orcutt[7] have shown that certain antibodies tend to become greatly concentrated within the udder. Howe[8] and others have shown that the colostrum of cows is rich in blood proteins. It is to be noted, however, that colostrum while rich in serum proteins usually reacts with serum precipitin to

[7] Little, R. B., and Orcutt, M. L., *J. Exp. Med.*, 1922, xxxv, 161.
[8] Howe, P. E., *J. Biol. Chem.*, 1922, lii, 51.

about one-half the titer of the blood serum itself. It may well be that certain proteins of the blood passing into the acini are reabsorbed, leaving a portion of the antigen within the udder. Thus a gradual accumulation of antigen may occur. Shortly after parturition the udder may be drained with a consequent marked fall in serum content. There, however, apparently exists for the next few days considerable permeability of the capillaries for serum proteins. This is borne out by the reaction of the milk to the serum precipitin, since it may react above the normal level for as long as 10 days or 2 weeks after parturition.

During the course of inflammation there may occur a considerable outpouring of blood protein. In the severe cases the exudate may give a titer at as great a dilution as the blood serum. In other milder cases where the inflammation may be confined only to the mucosa of the milk cystern, there is usually little that is abnormal in the appearance of the milk. The precipitin test, however, indicates that serum proteins are present in definitely greater concentration than in normal milk.

Of practical interest is the effect of heating the milk on the reaction of serum precipitin. Milk heated to a temperature as high as 66°C. for 20 minutes will react at the same dilutions as the raw milk. Milk pasteurized at 68–69°C. for 20 minutes will no longer react. The precipitin test, then, affords a ready means of detecting milk heated above 68–69°C.

It is assumed that the precipitin test may have some further practical application. The evidence presented indicates a well defined serum proportion in the milk which may be increased during inflammation of the udder. The experiments in which mastitis exudate was mixed with varying quantities of market milk indicate that such mixtures can readily be detected with serum precipitin. It seems not improbable that some such procedure might be applied as a presumptive test to the product of small herds or to the mixed milk of a number of cows.

THE TRANSFORMATION OF MONOCYTES INTO FIBROBLASTS THROUGH THE ACTION OF ROUS VIRUS.

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PLATES 9 AND 10.

(Received for publication, January 19, 1926.)

A few years ago, monocytes in pure culture were found to metamorphose under certain conditions into elongated or stellate cells, and to assume the appearance of fibroblasts from which they became indistinguishable.[1] The processes of the transformed cells were long and sharp, and did not show any of the movements that characterize the pseudopods of monocytes; their nucleus was oval with one or two nucleoli; their cytoplasm contained a number of granules that took up neutral red in the same manner as do the fibroblasts kept in pure culture for over 13 years. As no permanent strain of these cells was obtained, it was not quite certain that the monocytes had metamorphosed into true fibroblasts. But the reality of such a transformation has been demonstrated recently by Fischer,[2] who succeeded in obtaining a permanent strain of fibroblasts from a culture of leucocytes.

In a culture medium, Fischer placed side by side some leucocytes and a fragment of muscle that had been kept in cold storage for a month. A number of tests had previously shown that no fibroblasts grew from the muscle. The leucocytes surrounded the muscle fragment, and every 2 or 3 days the cultures were washed and transferred into a new medium. Generally some fibroblasts could be observed around the muscle after the fourth passage, and their number increased during the following days. Then, the tissues were placed in a medium containing a large amount of embryonic tissue juice. In this manner, a strain of fibroblasts was obtained which remained active *in vitro* permanently.

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[1] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1922, xxxvi, 365.

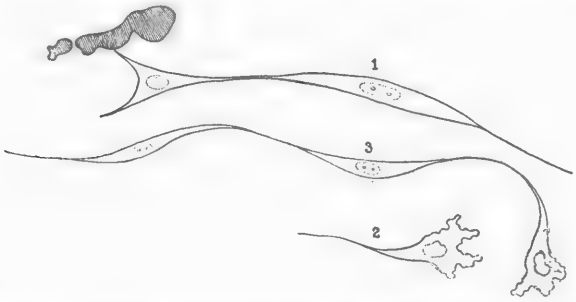
[2] Fischer, A., *Compt. rend. Soc. biol.*, 1925, xcii, 109.

The purpose of the present paper is to describe the conditions that bring about the transformation of the monocytes, to record the attempts to reproduce these conditions, and to give the results of a study of the characteristics of the cells.

Conditions of the Cultures That Bring About the Transformation.

When monocytes are cultivated in a flask instead of in a hanging drop, they never become transformed into fibroblasts. They wander through the medium, invade it progressively, and finally cover the whole area of the coagulum. They scatter themselves, never congregate in a mass, and never form a tissue. Generally, they completely fill the medium of a flask in 3 or 4 weeks. When the coagulum is removed from the flask and cut into fragments which are transferred into another flask, the cells migrate from the old coagulum and invade the new medium, but no fibroblasts ever appear. On the other hand, when leucocytes are cultivated on a cover-glass in a drop of plasma, according to the early technique, they may become transformed into fibroblasts. Although the composition of the medium is the same as in the flasks, the relations of the cells to the coagulum are quite different. The leucocytes are not left undisturbed for weeks, as in the experiments above described. Every few days, the part of the coagulum containing the cells is excised with a cataract knife, washed, and placed in a new medium. When the film that embeds the monocytes is sufficiently thick, when its edges are cut with a very sharp knife, and when they do not fold, the cells easily migrate into the new medium, and no transformation takes place, even when the cultures are kept for 3 months. But if, in the course of the experiments, the edges of the plasma film become slightly folded, or if the coagulum contracts, the cells, instead of migrating into the new medium, may accumulate along the obstacle that prevents their further migration. They congregate in amorphous masses of apparently dead tissue. When this condition arises, the transformation often begins. From the dark mass produced by the accumulation of the monocytes, some elongated and mobile processes start to grow, and fusiform cells appear (Text-fig. 1). At the same time, transition forms are observed. Some ameboid cells show active pseudopods, while one of the processes has become sharp, elongated, and fixed (Text-fig. 2). In some other cells,

two sharp nucleoli appear in the nucleus. There is also a marked tendency to tissue formation. It is well known that monocytes never unite. One of the first signs of the transformation is the union of cells through their processes, and their tendency to form a reticulum. In one culture, a chain of three cells was observed. Two of these cells were typical stellate fibroblasts, while the third one was a monocyte united by a slender process to a neighboring fibroblast (Text-fig. 3). These observations, which were repeated several times during our first study of the phenomenon, showed that the transformation takes place when the cells are packed together through some mechanical



TEXT-FIG. 1. Fusiform cells growing out from clump of dead monocytes.

TEXT-FIG. 2. Monocyte bearing active pseudopods and a fixed process at the same time.

TEXT-FIG. 3. Two fibroblasts and a transition cell united as a chain.

factors such as contraction of the medium, or tearing of its edges, which prevent their free migration, and determine their accumulation. Around the masses of apparently dead tissues, the transformation may begin. It seems as if the dying monocytes set free a substance which has the power of determining the metamorphosis of the other cells.

Attempt to Reproduce the Conditions Determining the Transformation.

The transformation of the monocytes around the fragments of dead tissue may be attributed to a change in the H ion concentration of the

medium, or to the setting free of some ferment or protein split products by the dying tissues. An attempt was made to reproduce these conditions artificially in several different ways.

Leucocytes were cultivated in media of pH varying from 5.5 to 8.5. When the medium was too acid or too alkaline, the leucocytes did not multiply. But in no case was a transformation observed.

Trypsin and the products of tryptic digestion of muscle were added to the culture. The monocytes did not change into fibroblasts.

Fragments of muscle, brain, spleen, liver, and leucocytic film killed by freezing were cultivated in flasks with living leucocytes. The leucocytes invaded the dead muscles and destroyed them in less than 3 weeks. But they did not become transformed into fibroblasts, although they crowded in immense numbers around the muscle fragments. It appeared that when the cells were free to migrate into the culture medium, the presence of muscle fragments did not determine the transformation. The discrepancies between the results of these experiments and those of Fischer must be attributed to differences in technique. In our experiments, the leucocytes were left undisturbed and could scatter through the entire medium of the flask, while in those of Fischer, the coagulum was excised and transferred to a new medium every few days. When this technique was used by us, the transformation sometimes occurred.

So far, the best method found for determining the fibroblastic transformation of a culture of monocytes is to add some filtered extract of Rous sarcoma to a culture of leucocytes. After a few days, some clumps of dead cells appear in the coagulum. Soon after, the digestion of the fibrin begins. The edges of the digested area are lined with small masses of apparently dead tissue. From these masses grow elongated cells that resemble fibroblasts. Generally they are full of fatty granulations and migrate very slowly into the medium (Fig. 1, *a*). When a fragment of culture is extirpated from the flask and kept in a hanging drop in a medium containing a large amount of tissue juice, the proliferation of these fibroblasts has been observed to continue for a few days. But their growth is far from being active. They resemble closely the fibroblasts from a spontaneous tumor of the chicken, observed some years ago by one of us, which had marked necrotic tendencies and killed the animal by cachexia rather than by metas-

tases.[3] They are far from being normal, as may be seen in the photograph (Fig. 1, *b*), and no permanent strain has been obtained so far.

Morphological Characteristics of the Cells.

The monocytes observed during the first hours of cultivation possessed the appearance that characterizes these cells and manifested the well known reaction to neutral red. The monocytes from a freshly prepared culture of leucocytes in diluted plasma show active pseudopods, and a nucleus that does not generally possess any definite nucleolus (Fig. 2, *a* and *b*). The granules stained by neutral red are small and move swiftly within the cells. They are scattered in the cytoplasm, without forming the rosette represented in the drawings of Sabin.[4] In the cultures that have been kept at a lower temperature, and where the cells are less active, the rosette becomes apparent. The length of these cells varies from about 10μ to 12 or 14μ. On the cinematomicrographic films, the cells are seen to progress rapidly, although they move much slower than the polymorphonuclears. Their mode of locomotion is analogous to that of the octopus. They differ markedly from the lymphocytes, which project short, blunt pseudopods and move slowly, or not at all. This cell is the element described under the names of monocyte, macrophage, endothelial leucocyte, blood histiocyte, or large mononuclear leucocyte, by various authors.

After a short time, the monocytes grow in size. They become from 80 to 140μ in length, and the nucleus is very much larger (Fig. 2, *d*). These giant cells devour with avidity the polymorphonuclears and the red blood corpuscles that are scattered in the coagulum. Soon, the medium contains only wandering monocytes. When stained with neutral red, they show small granules around the nucleus, which may also invade the ends of the cells but are not seen in the pseudopods. Generally the cytoplasm does not contain any unstained and refractile granules. The nucleus does not show any sharp nucleolus. The pseudopods are very active. Sometimes they resemble flagellates.

[3] Carrel, A., *Compt. rend. Soc. biol.*, 1924, xc, 1380.

[4] Sabin, F. R., Doan, C. A., and Cunningham, R. S., *Carnegie Institution of Washington, Pub. No. 361, Contributions to Embryology*, 1925, xvi, 125.

The cells divide freely, and show great phagocytic activity. The fibroblasts that appear in the cultures are of the same size as the giant monocytes. In the camera lucida drawing of a culture of leucocytes from the blood, treated by Rous virus, the fibroblasts are seen as elongated or stellate cells with a large oval nucleus containing one or two nucleoli (Fig. 2, *e*). The pseudopods have become transformed into apparently fixed processes. Small neutral red granules are located in the cytoplasm around the nucleus. At the periphery of the zone they occupy, more or less numerous refractile granules are observed. The red granules are more abundant and more deeply stained than in the fibroblasts described by Evans,[5] and their disposition closely resembles that found in the 13 year old strain of fibroblasts.

To summarize: The first change undergone by the monocytes is a considerable increase in their size. They become about ten times larger and their length equals that of normal fibroblasts. The giant monocytes possess the characteristics of tissue macrophages from which they are indistinguishable. They are the immediate precursors of the fibroblasts, and do not differ essentially from the cells that grow from a fragment of adult connective tissue.

Significance of the Transformation.

It is well known that one of the more important cultural characteristics of monocytes is their inability to form a tissue. When free to move in the medium, they always place themselves at a certain distance from one another. If they are packed together by centrifugation and embedded in a film of plasma, they migrate rapidly from the coagulum. When migration is impossible, they die. Fibroblasts, on the contrary, always live as a tissue. When they are in close contact, they multiply actively without scattering over the coagulum. They remain packed together. The peripheral cells of the growing colony are generally in contact with one another, or are united by their processes. It is important to observe that the transformation of monocytes into fibroblasts generally occurs when, on account of some change in the medium, the life of the monocytes has become impossible.

[5] Evans, H. McL., and Scott, K. J., *Carnegie Institution of Washington, Pub. No. 273, Contributions to Embryology*, 1921, x, 1.

The monocytes transform themselves into cells capable of living under the conditions present in the culture. The metamorphosis of a monocyte into a fibroblast displays the characteristic of an adaptive change which may automatically be produced by substances set free by the monocytes themselves under certain conditions.

The phenomenon that occurs in cultures of monocytes inoculated with Rous virus probably has the same significance. When an area of digestion occurs in the coagulum, masses of necrotic tissue appear along its edges. Around these masses, the fibroblastic forms develop. The monocytes that are highly susceptible to Rous virus become transformed into fibroblasts that are not sensitive to the virus, and that are not even a favorable medium for the growth of the virus. It seems as if there were a tendency for a susceptible cell to transform itself into an immune cell. This phenomenon could be considered as an expression of the general property with which all living organisms or chemical systems are endowed, that of opposing the action of a disturbing factor. But the real significance of these facts cannot be understood until we know whether the transformation of monocytes into fibroblasts is reversible.

SUMMARY.

In normal cultures, the transformation of monocytes into fibroblasts generally occurred when cells became packed together through some mechanical factors that prevented their free migration and determined their accumulation. Various modifications of the medium, the addition of dead tissue, and of trypsin or the products of trypsin digestion, failed to bring about the transformation. The inoculation of cultures of monocytes with filtered extract of Rous sarcoma frequently determined the appearance of fibroblasts. The first change undergone by the monocytes cultivated *in vitro* was a large increase in their size. Later, the giant monocytes became transformed into cells that did not differ essentially from those that grow from a fragment of adult connective tissue.

EXPLANATION OF PLATES.

PLATE 9.

FIG. 1, *a* and *b*. Fibroblasts observed in a culture of monocytes treated with filtered extracts of Rous sarcoma. (*a*) Fibroblasts united by their processes. (*b*) Fibroblasts containing fat granules.

PLATE 10.

FIG. 2, *a* to *e*. Normal monocytes after a few hours cultivation. Camera lucida drawing made 20 minutes after the cells were placed in contact with a 1/50,000 solution of neutral red. × 1055. The stained granules are represented by solid black, and the refractile bodies by black rings. (*a*) Very active monocytes. (*b*) Monocytes in less active condition; rosette disposition of the neutral red granules. (*c*) Red blood corpuscle. (*d*) Giant monocytes after 2 weeks cultivation. (*e*) Fibroblasts resulting from the transformation of the monocytes in a culture treated with filtered extract of Rous sarcoma.

FIG. 1.

(Carrel and Ebeling: Transformation of monocytes.)

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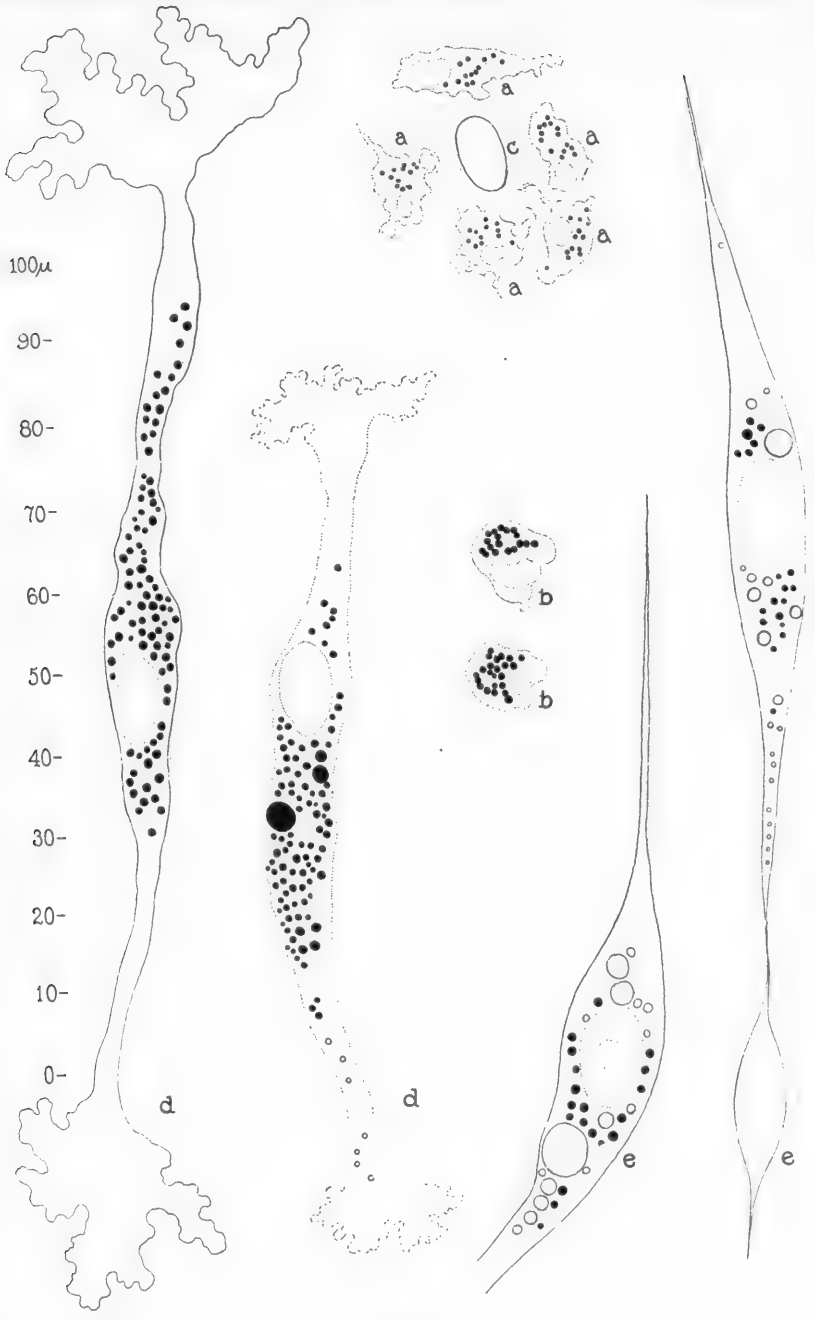

FIG. 2.

(Carrel and Ebeling: Transformation of monocytes.)

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ANAPHYLACTIC SHOCK CAUSED BY ANTIBODY IN ANIMALS SENSITIZED BY ANTIGEN—REVERSED PASSIVE ANAPHYLAXIS.

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Manifestations of hypersusceptibility which have received most attention are anaphylactic shock and the local inflammatory reaction produced by injection of antigen into a sensitized animal (Arthus phenomenon). Since these phenomena may be reproduced in a normal animal by injecting into it blood serum obtained from one which has been actively sensitized, it may be assumed that this susceptibility to the action of an otherwise harmless substance is caused by an antibody which finds its way into the blood serum of the sensitized animal.

One of us has found that the usual procedure employed to produce the Arthus phenomenon by means of passive sensitization may be reversed.[1] An animal previously treated with horse serum reacts with acute inflammation when serum of a rabbit immunized against horse serum is injected into its dermis. Inflammatory edema is caused by antibody injected into the skin of an animal sensitized by the corresponding antigen. This observation indicates that acute inflammation will occur whenever antigen and antibody come into contact within the tissues.

The experiments which will be described have been undertaken with the purpose of determining if the meeting of antigen and antibody is sufficient to produce anaphylactic shock irrespective of the order of their introduction into the body. It has seemed probable that further knowledge concerning the relationship of antigen to antibody might explain the pathogenesis of both local and general hypersensitiveness.

[1] Opie, E. L., *J. Immunol.*, 1924, ix, 255.

Experiments on Guinea Pigs.

Since guinea pigs have been most frequently used for the study of anaphylactic shock because of their peculiar susceptibility to it, experiments have been undertaken to determine if this animal is susceptible to reversed passive anaphylaxis as well. Doerr and Russ[2] were unsuccessful in efforts to produce anaphylactic shock by injection of anti-eel serum into guinea pigs which had received eel serum a short time before. Similar experiments were performed with anti-goat serum injected into guinea pigs which had received serum of goat.

In our own experiments anti-horse serum of guinea pig in quantity only slightly less than that which was fatal for normal animals caused when injected into animals previously treated with horse serum no symptoms of shock. It is noteworthy that the guinea pig which is highly sensitive to anaphylactic shock forms antibodies much less actively than the rabbit. The precipitin titre of the serum used in this experiment was very low. It is possible that a stronger serum might cause some reaction.

Since passive anaphylaxis is produced in guinea pigs more readily with serum of sensitized rabbits than with serum of sensitized guinea pigs, experiments were performed to determine if reversal of the usual procedure causes anaphylactic shock when serum of rabbits is used. These experiments have shown that the serum of immunized rabbits is much more toxic for normal guinea pigs than the serum of normal rabbits. Furthermore anti-horse, anti-beef, and anti-egg white serum of rabbit have been found no more toxic for guinea pigs which have previously received the corresponding antigen than for untreated guinea pigs.

Immune sera prepared by use of one mammalian serum such as horse serum or beef serum precipitates weakly sera derived from other more or less distantly related species and the possibility suggests itself by analogy that immune serum may react with protein of the guinea pig and cause anaphylactic shock. Nevertheless it is noteworthy that serum prepared by use of an antigen of avian origin, namely egg white, has been just as toxic as anti-horse or anti-beef serum. This serum has caused very slight precipitation when mixed with serum of

[2] Doerr, R., and Russ, V. K., *Z. Immunitätsforsch., Orig.*, 1909, iii, 706.

guinea pig. A second possibility may be considered. In the experiments on guinea pigs the immune serum which has been used has been collected from several animals repeatedly injected with antigen at intervals of 5 days. In some of these animals antigen may have persisted in the blood stream so that the injected serum has contained both antigen and antibody. Subsequent experiments have shown that mixtures of antigen and antibody may cause shock in rabbits.

Experiments on Rabbits.

Rabbits furnish more favorable conditions for the production of reversed passive anaphylaxis than guinea pigs. Antibody formation appears to be more active in the rabbit than in the guinea pig, for passive sensitization of guinea pigs is more readily produced with serum obtained from actively sensitized rabbits than with that obtainable from guinea pigs. Furthermore precipitin formation is much more active in rabbits than in guinea pigs. When the rabbit is used and rabbit serum with high antibody content is injected into the circulating blood, the experiment is not complicated by the toxicity of a foreign serum. Reversed passive anaphylaxis occurs in rabbits when a sufficient quantity of strong antiserum is introduced into the vascular system of animals previously treated with the corresponding antigen. Young rabbits have been used in the experiments because it has been found impracticable to obtain antiserum in quantity sufficient to inject several fully grown animals.

Rabbits received 5 cc. of horse serum injected into the peritoneal cavity and on the next day anti-horse serum of rabbit obtained from four animals was injected into the ear vein. The injection in all experiments has been timed so that the serum entered the vein at the rate of 10 cc. per minute.

Rabbit No.	Weight.	Horse serum.	Interval.	Anti-horse serum.	Result.
	gm.	*cc.*	*hrs.*	*cc.*	
1	420	5	20	5	Shock.
2	510	5	20	7.5	"
3	580	5	20	9	Death.
4	500	5	20	10	"
5	420	—	—	10	No symptoms.

Animals which have received 5 cc. of horse serum and 20 hours later

from 5 to 10 cc. of anti-horse serum have exhibited symptoms of shock or have died immediately following the second injection, whereas a control animal which has received no preliminary injection of horse serum has shown no symptoms when injected with anti-horse serum.

In the animals in which shock has occurred there have been passage of urine and feces and weakness following the injection so that the animal has rested upon the abdomen with the legs spread out and flaccid; respiration has been slow and often labored. Recovery has occurred after 10 or 15 minutes. In the animals which have died there have been convulsive extension of the legs, passage of urine and feces, dyspnea with slow respiration, and death within 4 or 5 minutes. The liver has been found to be engorged with blood and the veins of the intestine have been congested. When "slight shock" is recorded there has been transient weakness of the extremities; with "severe shock," convulsions or prolonged prostration with almost complete loss of reflexes has been followed by recovery.

In a second experiment a smaller quantity of horse serum was injected into the peritoneal cavity.

Rabbit No.	Weight.	Horse serum.	Anti-horse serum.	Result.
	gm.	*cc.*	*cc.*	
6	300	1	5	No symptoms.
7	320	1	10	Shock.
8	320	1	15	Death.
9	300	—	10	No symptoms.
10	320	—	15	Slight shock.

Preliminary injection of horse serum has sensitized rabbits to the action of anti-horse serum.

Full grown rabbits have been found to be susceptible to the changes observed in young animals. Anti-horse serum has been injected into the ear vein of rabbits which have previously received 5 cc. of horse serum intravenously.

Rabbit No.	Weight.	Horse serum.	Anti-horse serum.	Result.
	gm.	*cc.*	*cc.*	
11	2170	5	10	Slight shock.
12	2400	5	20	No symptoms.
13	2400	5	30	Death.

In Rabbit 13 death occurred preceded by convulsions, passage of urine and feces, and dyspnea. The liver was engorged and the vessels of the intestine were injected. There was delayed coagulability of the blood. Other experiments have shown that the quantity of antiserum used in this experiment has no injurious effect.

Sensitizing Dose of Antigen.

The amount of antigen used as a preliminary injection has been varied to determine if sensitization to antiserum shows any corresponding variation. Five typical experiments with five samples of antiserum are as follows:

Rabbit No.	Weight.	Horse serum.	Interval.	Anti-horse serum of rabbit.	Result.
	gm.	*cc.*	*hrs.*	*cc.*	
14	300	5	20	8	Shock.
15	300	0.2	20	8	Severe shock.
16	290	—	—	8	No symptoms.
17	220	0.5	13	9	Severe shock.
18	220	0.05	13	8	Slight "
19	230	0.01	13	9	" "
20	200	—	—	8	No symptoms.
21	190	—	—	10	" "
22	320	2	20	15	Severe shock.
23	280	0.1	20	20	Shock.
24	270	0.02	20	15	No symptoms.
25	280	0.002	20	15	" "
26	210	2	20	9	Shock.
27	350	0.5	20	12	Slight shock.
28	200	0.2	20	9	Shock.
29	320	—	—	13	Slight shock (?).
30	330	—	—	15	No symptoms.
31	285	0.5	20	9	Death.
32	285	0.05	20	8	"
33	285	0.005	20	8	No symptoms.

These experiments show that variation of the amount of antigen

between 2 and 0.05 cc. has no constant effect upon the changes caused by a subsequent injection of antiserum but smaller quantities of antigen have been followed by less severe symptoms and 0.005 cc. has been found insufficient to produce any sensitization to antiserum.

Toxic Dose of Antibody.

In Rabbit 32 death was caused by 8 cc. of antiserum following sensitization by 0.05 cc. of horse serum; this is the smallest quantity of antiserum which has produced maximum intoxication. Sensitization has been demonstrated by injection of anti-horse serum in volume 180 times that of antigen. This figure accords approximately with the quantitative relation of antigen to antiserum in the precipitin reaction. The amount of antiserum necessary to produce death in animals sensitized by antigen has varied from 8 to 20 cc. and with the same serum may vary with the weight of the animal.

Interval between Injection of Antigen and Antibody.

When anaphylaxis is produced by the usual method an interval must elapse between the sensitizing injection of antiserum and the toxic injection of antigen. It is assumed that this period of incubation is required to permit penetration of antibody in sufficient concentration into cells upon which antigen acts when it produces symptoms of shock. Experiments were made to determine if passive sensitization is preceded by a similar interval when antigen is followed by antiserum; that is, when their usual order of introduction is reversed.

Four experiments are cited to show the effect of varying the interval between the sensitizing injection of antigen and the subsequent injection of antiserum. The second injection is said to be "immediate" when the first injection is made into the marginal ear vein on one side and is immediately followed by injection of antiserum into the same vein of the other ear, the interval between the two injections being less than 30 seconds.

Rabbit No.	Weight.	Horse serum.	Interval.	Anti-horse serum of rabbit.	Result.
	gm.	*cc.*	*hrs.*	*cc.*	
34	400	1	20	15	Death.
35	390	1	20	10	"
36	400	1	12	12	Severe shock.
37	400	1	6	12	Death.
38	550	1	Immediate.	17	No symptoms.
39	420	—	—	15	" "
22	220	0.5	13	9	Severe shock.
40	200	0.5	Immediate.	9	Slight "
25	200	—	—	8	No symptoms.
26	190	—	—	10	Transient weakness.
41	340	0.5	20	10	Death.
42	390	0.5	20	15	"
43	360	0.5	2	11	No shock.
44	380	0.5	Immediate.	15	Death.
45	410	0.5	"	12	Transient weakness.
46	340	—	—	15	" "
Rabbit No.	**Weight.**	**Beef serum.**	**Interval.**	**Anti-beef serum.**	**Result.**
	gm.	*cc.*	*hrs.*	*cc.*	
47	720	1	20	20	Death.
48	770	1	6	20	Transient weakness.
49	770	1	Immediate.	20	" "

Experiments have been performed on small groups the size of which has been determined by the amount of available antiserum. In each group there have been from one to three animals in which the interval between the sensitizing injection of antigen and the subsequent toxic dose of antiserum has been from 12 to 20 hours and in each group there have been one or more controls in which injection of antiserum has not been preceded by injection of antigen. Furthermore, these groups included animals in which the period between the sensitizing and toxic injection has varied from less than 30 seconds ("immediate") to 6 hours. The following table includes all of the animals which have received 0.1 cc. or more of antigen and antiserum in sufficient quantity to produce definite symptoms when the interval between injection of antigen and antibody has been from 12 to 20 hours.

	Number of animals which died.	Number with severe shock.	Number with moderate shock.	Number with slight shock.	Number with no symptoms.
Controls (no injection of antigen).....	1	1	1	7	14
Interval between injection of antigen and of antiserum.					
Immediate.....	1	1	1	4	3
2 hrs.....	1	—	—	2	—
4 "	1	—	3	3	—
6 "	1	—	—	1	—
12 "	2	3	—	1	—
24 "	15	8	6	1	—

When control animals have been injected with antiserum alone, in most instances there have been no symptoms but in a few instances shock has occurred. It will be shown below that this shock is probably referable to the presence of antigen in the antiserum used for injection.

When injection of horse serum into one ear vein is followed immediately by injection of anti-horse serum into a vein of the opposite ear mild shock occurs in most instances but is much less frequent than in those instances in which an interval of 4 or more hours elapses between the two injections. In animals in which the interval between injection of antigen and of antiserum has been from 2 to 6 hours shock has occurred constantly but has been much less severe than in animals in which the interval has been from 12 to 20 hours. The experiments show that the intensity of sensitization increases gradually from the time of injection and reaches a maximum after 4 hours. After 12 hours there is no further increase.

In the greater part of nineteen experiments anti-horse or anti-beef serum injected into the ear vein of untreated rabbits has caused no symptoms but in five instances there has been transient symptom of shock and in an occasional instance definite shock or death. Experiments of Friedemann,[3] Briot,[4] and others, have shown that mixtures of antigen and antiserum injected into the ear vein of rabbits may cause anaphylactic shock. Under the conditions of the fore-

[3] Friedemann, U., *Z. Immunitätsforsch., Orig.*, 1909, ii, 591.

[4] Briot, A., *Compt. rend. Soc. biol.*, 1910, lxviii, 402.

going experiments it is not improbable that the injected antiserum in some instances contained antigen. In the early stages of immunization against horse or beef serum, the antigen makes its appearance in the serum of the blood but with continued immunization it fails to enter the blood.[5] Nevertheless in a few animals in which precipitin formation is scant antigen may persist in the blood serum. In the experiments which have been described, serum from three or four or more animals has been mixed in order to obtain a sufficient quantity of serum. Antigen has doubtless been present in some of the mixtures of serum injected into normal animals.

Rabbit 50, which had received no preliminary injection of beef serum, received in the ear vein 20 cc. of anti-beef serum obtained from four rabbits. Following slight restlessness, urine and feces were passed. The animal became very weak and lay with legs outstretched. The respiration was slow and labored. Recovery occurred after about 20 minutes. The mixture of serums used for injection of this animal formed on standing a flocculent sediment. This precipitate was removed by centrifugalization and 20 cc. of the serum were injected into the ear vein of a rabbit. Slight transient weakness of the extremities was noted. Part of the serum used for this injection became slightly turbid on standing.

Rabbits 67 and 68 received in the ear 12 cc. of anti-horse serum obtained from four rabbits. In Rabbit 67 the injection was followed by weakness of the extremities so that the animal lay with extremities outstretched and head resting on the table. Recovery occurred within 5 minutes. In Rabbit 68 there were transient weakness of extremities and slow respiration. Three of the specimens of serum used contained no antigen whereas the fourth contained antigen in abundance so that the mixture of the four formed an abundant precipitate. When this precipitate was removed by centrifugalization the clear serum caused no symptoms when 12 cc. were injected into the ear vein of each of two rabbits.

In these experiments serum containing both antigen and antibody has caused shock but when all of the antigen has been removed there has been no evidence of shock following injection of the serum.

The following experiment in which a strong anti-egg serum has been used suggests that the interval between injection of egg white and maximum sensitization to anti-egg serum may be shorter than the interval in animals tested with horse or beef serum and the corresponding antiserum. The serum employed in this experiment was obtained from four rabbits, had a precipitin titre of one million, and contained no antigen.

[5] Opie, E. L., *J. Immunol.*, 1923, viii, 55.

Rabbit No.	Weight.	Egg white.	Interval.	Anti-egg white serum.	Result.
	gm.	*cc.*	*hrs.*	*cc.*	
51	370	0.5	24	25	No symptoms.
52	300	0.45*	24	20	" "
53	360	0.45*	7	20	Slight shock.
54	350	0.5*	4	20	" "
55	310	0.5	Immediate.	20	No symptoms.
56	310	—	—	25	" "

* These animals had received on the previous day 0.25 cc. of egg white, diluted with an equal volume of salt solution.

In the experiment maximum sensitization has been present at 4 and 7 hours after injection of egg white and no evidence of sensitization has been found immediately after injection and after an interval of 24 hours.

Desensitization.

The parallel between reversed passive anaphylaxis and the usual procedure for the production of passive anaphylaxis is emphasized by the occurrence of desensitization. Animals which have been sensitized to the action of anti-horse serum of rabbits by an injection of horse serum may be desensitized by repeated injection of anti-horse serum in quantity insufficient to produce symptoms. Young rabbits have been given the smallest quantity of antigen required to produce effective sensitization to antiserum. On the day following the injection of antigen antiserum in quantity much below that which produces symptoms of shock has been given into the ear vein; from 30 minutes to 1 hour later, approximately half the usual dose required to produce severe shock or death has been administered. Several hours later the full toxic dose has been given. For comparison a sensitized rabbit which has received no preliminary injections of anti-horse serum has been treated with the same toxic dose.

Rabbit No.	Weight.	Horse serum.	Interval.	Anti-horse serum.	Interval.	Anti-horse serum.	Interval.	Anti-horse serum.	Result.
	gm.	*cc.*	*hrs.*	*cc.*	*min.*	*cc.*	*hrs.*	*cc.*	
57	260	0.05	20	2	30	7.5	3	15	No symptoms.
58	260	0.05	20	—	30	—	3	15	Shock.

In this experiment 15 cc. of anti-horse serum administered to rabbits sensitized by horse serum have caused shock with passage of urine and feces, very slow respiration, profound weakness, and partial loss of reflexes whereas intravenous injection of antiserum in quantity insufficient to produce symptoms has caused complete desensitization so that 15 cc. of anti-horse serum have caused no symptoms.

To exclude the possibility that serum alone might cause desensitization in subsequent experiments an equal quantity of normal serum has been injected whenever anti-horse serum has been given with the purpose of causing desensitization.

Rabbit No.	Weight.	Horse serum.	Interval.	Anti-horse serum.	Interval.	Anti-horse serum.	Interval.	Anti-horse serum.	Result.
	gm.	*cc.*	*hrs.*	*cc.*	*min.*	*cc.*	*hrs.*	*cc.*	
59	230	0.1	20	2	45	7.5	4	15	No symptoms.
60	200	0.1	20	—	45	—	4	15	Slight shock.
					hrs.				
61	350	0.1	20	2	1	7	3½	13	No symptoms.
62	360	0.1	20	—	1	—	3½	13	Severe shock.
63	320	0.1	20	2	1	6	3½	14	No symptoms.
64	320	0.1	20	—	1	—	3½	14	Shock.
65	230	0.1	20	2	1	8	3	20	No symptoms.
66	230	0.1	20	—	1	—	3	20	Severe shock.

Under the conditions which have been described desensitization has occurred in all experiments.

DISCUSSION.

The experiments which have been described show that anaphylactic shock may occur when the usual procedure employed for passive sensitization is reversed. In rabbits which have received antigen the corresponding antiserum in sufficient quantity causes anaphylactic shock and death. It has been shown that the reaction occurs under conditions which reproduce those of passive anaphylaxis. An interval of approximately 4 hours must intervene between injection of antigen and injection of antiserum in order to produce maximum shock; ani-

mals sensitized by horse serum may be desensitized to the action of anti-horse serum by repeated injection of anti-horse serum in quantity insufficient to cause symptoms. Anaphylactic shock like the specific inflammatory reaction of the immunized animal or Arthus phenomenon occurs when antigen and antibody meet and in either instance the usual order of their introduction may be reversed. In the one instance they meet within the tissue spaces whereas in the other contact occurs by way of the circulating blood.

The occurrence of an interval between the injection of a sensitizing substance (antibody or antigen) and the appearance of maximum sensitization furnishes evidence in favor of the view that the phenomena of anaphylaxis are referable to changes which occur within the cells. It has been assumed that with passive sensitization, antibodies penetrate from the blood stream into the cells of the body so that after an interval of approximately 4 hours they have reached maximum concentration within the cytoplasm. The tissue is thus prepared for action of antigen. The experiments described in this paper have shown that tissues may be prepared just as readily by antigen and then stimulated by antibody. These experiments and similar observations upon the Arthus phenomenon indicate that the phenomena of general and local anaphylaxis occur whenever antigen and antibody meet in sufficient concentration within the tissues, the resulting changes being dependent upon the peculiar functions of the affected cells and their susceptibility to stimulation or injury by antigen and antibody.

There is no direct evidence to show how antigen and antibody, meeting within smooth muscle fibres, bring about contraction as with anaphylactic shock, or meeting perhaps within the endothelial cells of vessel walls increase their permeability for fluid and cells as in the Arthus phenomenon. When antigen and the corresponding antiserum are brought together a precipitate is formed but with existing knowledge it is not possible to determine the relation of precipitin to the antibody concerned in the production of anaphylactic shock or of the specific inflammatory reaction known as the Arthus phenomenon. Nevertheless there is no longer any reason for doubting that precipitation occurs within the body as well as *in vitro* whenever antigen and the corresponding precipitin meet; the reaction is identical when dilu-

tions are made with blood serum or with salt solution. The close relation which exists between susceptibility to anaphylactic shock and precipitin has been pointed out by several observers and susceptibility to the Arthus phenomenon has been found to bear a close if not exact relation to the precipitin content of the serum.

The investigations which have been cited show that local anaphylaxis (susceptibility to specific inflammation or Arthus phenomenon) occurs under conditions identical with those which induce general anaphylaxis (susceptibility to anaphylactic shock) save that in the first instance antigen and antibody are brought together within the tissue spaces outside of blood vessels and cause the usual phenomena of inflammation whereas in the latter instance one or other of the two agents is introduced by way of the circulating blood and has the opportunity of coming into contact with those tissues which after preparation by the other agent are most susceptible to the two in combination. Should precipitin be the antibody concerned in the production of local or general anaphylactic reactions it is essential that precipitin and precipitinogen meet and react to form precipitate within the tissue for the introduction of the precipitate formed by their union causes neither local nor general anaphylaxis. There is no reason to doubt that precipitin and precipitinogen meeting within the cytoplasm of smooth muscle fibre or of endothelial cell would form precipitate and it is not improbable that the presence of this precipitate within the cell would produce disturbances such as muscular contraction in one instance and increased permeability in the other.

It is unnecessary to assume the sudden formation of a toxic substance or anaphylatoxin with the power to elicit the symptoms of anaphylactic shock. Toxic fluids which reproduce these symptoms have been formed *in vitro* by the prolonged action of normal serum (containing complement) upon a combination of antigen and antibody, for example upon sensitized red blood corpuscles or upon precipitate formed by precipitinogen and precipitin. The formation of toxic substances by the action of various substances such as kaolin, peptone, agar, etc., upon blood serum does not explain the changes of local or general anaphylaxis for the phenomena of anaphylaxis are caused by the meeting of antigen and antibody.

CONCLUSIONS.

Anaphylactic shock occurs (in rabbits) when the usual procedure for the production of passive anaphylaxis is reversed; that is, when an animal previously treated with antigen receives the corresponding antiserum by way of the circulating blood.

This susceptibility to the action of anti-horse serum produced by injection of antigen reaches maximum intensity after an interval of 4 hours presumably required to permit penetration of the antigen in sufficient concentration into the tissues.

Desensitization to the action of a shock-producing dose of anti-horse serum can be brought about by repeated small doses of the same antiserum.

Anaphylactic shock and local anaphylaxis manifested by the acute inflammation of an immunized animal when injected with the antigen used for immunization (Arthus phenomenon) occur under analogous conditions; that is, when antigen and antibody meet within the tissues. The peculiar characters of these reactions are dependent upon the site of entry of the irritating agent, which is the vascular system in one instance and tissue spaces in the other, and upon the concentration of antigen and antibody within susceptible tissues.

Meeting of antigen and antibody within susceptible tissues is sufficient to explain the phenomena of local and general anaphylaxis so that it is unnecessary to assume the sudden formation of a toxic substance (anaphylatoxin).

EFFECT OF JEJUNOSTOMY IN EXPERIMENTAL OBSTRUCTION OF THE JEJUNUM OF THE DOG.

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Jejunostomy has been used in the clinical treatment of acute intestinal obstruction with great apparent benefit. Summers (1), Mayo (2), Long (3), and others have reported good results and recommend its use highly. The following experiments have been made to determine the effect of experimental jejunostomy on dogs having obstruction of the jejunum.

In previously published studies we have reported the chemical changes in the blood and urine observed in pyloric (4) and high intestinal obstruction (5) and have emphasized the therapeutic value of sodium chloride solution in such conditions (6). In the series of experiments tabulated below, we have studied the effect of jejunostomy upon the chemical changes of the blood and the duration of life of dogs with high jejunal obstruction. The effect of combined jejunostomy and treatment with sodium chloride is also noted.

Method.

Dogs were used for all experiments. The obstruction was produced under ether anesthesia and the secondary jejunostomy was done with local anesthesia, ½ per cent novocaine being used. The obstruction was made 10 to 12 inches below the ligament of Treitz by sectioning the gut and invaginating the ends. A small rubber tube of uniform size was used in the jejunostomy. The technique of jejunostomy was a modified Witzel operation with an omental covering sutured over the area. In every instance drainage through the tube was prompt and continuous. Water was permitted *ad libitum*. Blood for analysis was withdrawn from the jugular vein daily. All animals showing complications at autopsy were rejected. The non-protein nitrogen was determined by the method of Folin and Wu (7), the urea nitrogen by the Van Slyke and Cullen (8) modification of the Marshall method, and the carbon dioxide-combining power by the method of Van Slyke and Cullen (9). The chlorides were determined on the tungstic acid filtrate in the manner suggested by Gettler (10).

TABLE I.

Simple Obstruction of Jejunum.

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Chlorides.	CO_2-combining power.	Treatment.
		mg.	*mg.*	*mg.*	*vol. per cent*	
1	0	24.6	9.1	520	35.3	Obstruction of jejunum.
	1	24.0	12.6	480	43.8	
	2	27.3	7.0	470	42.8	
	3	33.3	23.8	490	38.1	
	4	26.1	14.0	490	41.9	
	5	25.0	9.1	450	43.8	
	6	23.6	11.9	420	52.0	
	7	33.7	14.7	410	55.8	
	8	31.2	11.9	390	59.6	
	9	68.3	44.1	360	53.9	
	10	175.0	85.4	310	34.3	Died.
2	0	34.9	13.3	480	39.0	Obstruction of jejunum.
	1	38.5	9.8	430	35.3	
	2	29.7	7.0	390	44.7	
	3	32.6	7.0	380	15.3	
	4	31.6	6.3	370	46.6	
	5	25.9	4.2	360	54.7	
	6	35.3	11.2	350	41.9	
	7	31.6	10.5	290	53.0	
	8	45.0	23.1	340	61.5	
	9	34.9	11.2	330	50.2	
	10	40.0	11.2	310	67.1	
	11	41.6	13.3	310	70.9	
	12	50.8	11.2	310	73.8	
	13	42.8	21.0	290	54.9	
	14	33.0	14.7	290	59.6	
	15	81.5	33.6	300	67.1	
	16	54.6	21.0	300	69.0	
	17	63.5	30.1	280	78.5	
	18	75.0	42.0	260	67.1	
	19	137.0	67.9	200	71.9	Died.
3	0	37.5	11.9	500	29.4	Obstruction of jejunum.
	1	20.0	7.7	430	41.9	
	2	33.3	7.0	410	32.4	

TABLE I—*Concluded.*

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Chlorides.	CO_2-combining power.	Treatment.
		mg.	*mg.*	*mg.*	*vol. per cent*	
3	3	31.9	4.9	410	43.8	
	4	28.5	4.2	400	43.5	
	5	46.0	27.3	370	40.9	
	6	32.6	8.4	340	40.9	
	7	30.1	11.9	360	57.7	
	8	32.6	11.9	350	58.7	
	9	36.6	11.2	330	59.6	
	10	36.1	14.7	310	50.2	
	11	38.9	11.9	330	64.3	
	12	58.8	27.9	270	44.7	Died.
4	0	37.0	14.7	500	33.4	Obstruction of jejunum.
	1	40.0	11.9	430	46.6	
	2	41.0	14.7	400	48.3	
	3	89.8	29.4	330	62.4	
	4	73.5	37.8	320	60.2	
	5	195.0	85.4	330	40.9	Died.
5	0	30.9	11.9	490	32.4	Obstruction of jejunum.
	1	39.5	8.4	450	39.0	
	2	34.5	7.0	420	34.3	
	3	38.9	5.6	400	41.9	
	4	32.3	15.4	400	48.2	
	5	79.0	42.0	400	48.2	Died.

EXPERIMENTAL OBSERVATIONS.

Five animals with simple obstruction of the jejunum lived from 5 to 19 days. The chemical changes of the blood were similar to those noted in duodenal obstruction, but developed less rapidly. The carbon dioxide-combining power shows an increase but not the marked rise observed in obstruction of the duodenum and pylorus (Table I).

In a second series of five animals in which obstruction of the jejunum and jejunostomy were done simultaneously the length of life varied

from 3 to 5 days. The chemical changes of the blood developed rapidly and were typical of those found in high intestinal obstruction (Table II).

TABLE II.

Obstruction of Jejunum and Jejunostomy Simultaneously. No Treatment.

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Chlorides.	CO_2-combining power.	Treatment.
		mg.	*mg.*	*mg.*	*vol. per cent*	
6	0	41.0	15.4	500	28.7	Obstruction of jejunum and jejunostomy.
	1	29.1	14.7	450	15.5	
	2	38.0	17.1	370	30.5	
	3	50.8	27.3	310	15.5	
	4	101.5	65.8	270	22.1	Died.
7	0	21.4	6.1	490	21.1	Obstruction of jejunum and jejunostomy.
	1	36.1	10.5	420	20.2	
	2		19.6	400	11.7	
	3	60.0	38.5	390	18.3	Died.
8	0	45.0	7.0	490	26.8	Obstruction of jejunum and jejunostomy.
	1	42.8	7.9	470	41.9	
	2	32.6	9.8	430	40.9	
	3	33.0	7.9	390	42.8	
	4	37.5	17.3	350	44.7	
	5	150.0	58.8	290	39.0	Died.
9	0	36.6	7.5	470	40.9	Obstruction of jejunum and jejunostomy.
	1	38.5	9.3	410	38.1	
	2	65.0	25.2	470	42.8	
	3	131.0	58.8	380	24.7	Died.
10	0	39.5	5.1	500	28.7	Obstruction of jejunum and jejunostomy.
	1	40.0	10.7	490	24.6	
	2	32.3	9.8	420	37.2	
	3	68.3	32.9	370	26.8	Died.

Animals subjected to obstruction of the jejunum and jejunostomy simultaneously and treated with sodium chloride solution from the

TABLE III.

Obstruction of Jejunum and Jejunostomy Simultaneously. Sodium Chloride Solution Given from Beginning of Experiment.

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Chlorides.	CO_2-combining power.	Treatment.
		mg.	*mg.*	*mg.*	*vol. per cent*	
11	0	34.9	19.6	450	35.3	Obstruction of jejunum and jejunostomy. 40 cc. per kg. of 1% NaCl subcutaneously.
	1	62.5	32.8	500	40.0	" " " " " 1 " " "
	2	37.0	18.2	470	38.1	" " " " " 1 " " "
	3	31.6	24.5	520	40.0	No injection.
	4	59.0	25.9	450	40.9	40 cc. per kg. of 1% NaCl subcutaneously.
	5	38.6	23.1	580	43.8	No injection.
	6	41.2	25.2	490	33.4	" "
	7	48.6	29.5	480	37.2	" " Died.
12	0	23.6	5.6	490	39.0	Obstruction of jejunum and jejunostomy. 40 cc. per kg. of 1% NaCl subcutaneously.
	1	24.6	9.1	480	33.4	" " " " " 1 " " "
	2	28.9	9.1	470	30.5	" " " " " 1 " " "
	3	37.0	11.2	530	43.8	No injection. Died.
13	0	38.5	14.0	510	36.2	Obstruction of jejunum and jejunostomy. 40 cc. per kg. of 1% NaCl subcutaneously.
	1	37.0	11.9	480	42.8	" " " " " 1 " " "
	2	25.7	8.4	440	52.0	" " " " " 1 " " "
	3	22.9	7.7	420	56.8	" " " " " 1 " " "
	4		7.0	460	49.2	" " " " " 1 " " "
	5	30.0	15.4	460	39.0	" " " " " 1 " " "
	6	35.3	17.5	520	38.1	No injection. Died.
14	0	29.4	5.6	530	34.3	Obstruction of jejunum and jejunostomy. 40 cc. per kg. of 1% NaCl subcutaneously.
	1	33.7	11.2	490	29.4	" " " " " 1 " " "
	2	29.7	12.6	490	30.5	" " " " " 1 " " "
	3	37.0	12.6	490	34.3	" " " " " 1 " " "
	4	39.5	15.4	500	27.5	" " " " " 1 " " "
	5	70.0	35.0	510	22.0	" " " " " 1 " " "
	6	147.0	74.2	500	23.8	" " " " " 1 " " " Died.

TABLE III—*Concluded.*

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Chlorides.	CO_2-combining power.	Treatment.
		mg.	*mg.*	*mg.*	*vol. per cent*	
15	0	30.0	10.5	480	30.5	Obstruction of jejunum and jejunostomy.
						40 cc. per kg. of 1% NaCl subcutaneously.
	1	22.8	5.6	470	36.2	" " " " " 1" " "
	2	27.0	10.5	480	37.2	" " " " " 1" " "
	3	29.4	14.0	500	33.4	" " " " " 1" " "
	4	34.1	11.9	530	39.1	" " " " " 1" " "
	5	30.9	11.2	520	40.9	" " " " " 1" " "
	6	30.9	10.5	510	40.0	" " " " " 1" " "
	7	41.6	14.0	490	37.2	" " " " " 1" " "
	8	38.0	16.8	460	50.2	" " " " " 1" " "
	9	37.0	18.2	450	43.8	" " " " " 1" " "
	10	53.6	24.5	500	39.0	" " " " " 1" " "
	11	65.2	25.2	540	40.0	" " " " " 1" " "
	12	60.0	30.8	510	34.3	" " " " " 1" " "
						Died.

beginning of the experiment lived from 3 to 12 days. They did not show the chemical changes in the blood characteristic of jejunal obstruction. The administration of sodium chloride solution apparently prevented such changes (Table III).

In five dogs with obstruction of the jejunum jejunostomy was done after the development of a toxemia. The duration of life in the group varied from 4 to 13 days. The animals developed the characteristic blood changes of simple jejunal obstruction (Table IV).

In the last series of five dogs jejunostomy was done after the onset of the toxemia of jejunal obstruction. Sodium chloride was given after jejunostomy in sufficient quantity to keep the blood chlorides within normal limits. These animals did not develop the chemical changes of the blood characteristic of obstruction of the jejunum and lived from 13 to 18 days (Table V).

TABLE IV.

Obstruction of Jejunum with Jejunostomy Later. No Sodium Chloride Solution Given.

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Chlorides.	CO_2-combining power.	Treatment.
		mg.	*mg.*	*mg.*	*vol. per cent*	
16	0	30.0	7.5	510	26.8	Obstruction of jejunum.
	1	25.9	10.5	430	23.0	
	2	25.7	8.4	400	28.7	
	3	25.7	7.7	400	24.9	
	4	22.0	4.2	400	32.4	
	5	25.9	4.2	430	38.1	
	6	18.3	4.0	420	38.1	
	7	16.6	7.0	490	33.3	
	8	18.3	7.7	370	43.8	
	9		9.8	310	32.4	
	10		15.4	340	36.2	
	11	31.6	11.2	330	51.3	Jejunostomy.
	13	154.6	77.7	300	32.4	Died.
17	0	33.0	11.2	460	25.8	Obstruction of jejunum.
	1	30.9	11.2	460	32.4	
	2	30.0	9.8	400	41.9	
	3	25.9	9.8	380	38.1	Jejunostomy.
	4	33.0	11.2	410	38.1	
	5	34.1	14.7	370	46.6	
	6	31.9	19.6	330	52.2	
	7	61.0	28.6	320	55.1	
	8	111.0	61.6	320	41.9	Died.
18	0	31.6	9.8	480	40.0	Obstruction of jejunum.
	1	40.0	9.3	440	43.8	
	2	39.5	11.2	410	43.8	
	3	36.1	7.9	420	46.6	
	4	30.9	13.2	390	47.5	Jejunostomy.
	5	35.3	14.0	370	48.5	
	6	42.8	21.7	340	47.5	
	7	37.5	14.7	350	46.6	
	8	47.6	23.8	370	50.2	
	9	80.8	47.6	330	51.7	
	10	171.0	66.5	320	57.7	

TABLE IV—*Concluded.*

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Chlorides.	CO_2-combining power.	Treatment.
		mg.	*mg.*	*mg.*	*vol. per cent*	
18	11	121.0	64.4	310	50.2	
	12	140.0	91.1	290	39.0	
	13	185.0	98.1	250	34.3	Died.
19	0	28.9	11.9	500	35.3	Obstruction of jejunum.
	1	28.5	13.3	450	40.0	
	2	37.5	13.3	410	33.4	
	3	30.0	18.9	400	41.9	
	4	27.0	14.0	400	48.3	
	5	38.9	15.4	330	36.2	
	6	28.5	15.4	350	52.0	Jejunostomy.
	7	62.5	42.7	350	45.7	
	8	140.0	79.1	310	40.9	Died.
20	0	41.0	14.7	490	34.3	Obstruction of jejunum.
	1	40.0	22.4	410	50.2	
	2	46.8	19.6	370	59.6	
	3	55.4	21.1	340	43.8	Jejunostomy.
	4	140.0	67.9	310	44.7	Died.

DISCUSSION.

In obstruction of the jejunum, the average duration of life without treatment is somewhat longer than in obstruction of the duodenum. The rise in the non-protein and urea nitrogen and the fall in the chlorides occur in both types of obstruction. The carbon dioxide-combining power increases less than in obstruction higher up.

The dogs having jejunal obstruction and jejunostomy simultaneously live a much shorter time than those having obstruction only. The jejunostomy does not prevent the occurrence of the chemical changes in the blood observed in jejunal obstruction. If, after the same operative procedure, animals are treated with sodium chloride as shown in the third series, duration of life is somewhat lengthened

TABLE V.

Obstruction of Jejunum with Jejunostomy Later. Sodium Chloride Solution Given after Jejunostomy.

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Chlorides.	CO_2-combining power.	Treatment.
		mg.	*mg.*	*mg.*	*vol. per cent*	
21	0	42.8	11.7	520	30.5	Obstruction of jejunum.
	1	39.5	18.9	450	40.0	
	2	35.7	16.3	450	40.9	
	3	35.3	16.8	450	39.0	
	4	58.5	27.3	400	49.2	
	5	60.0	19.6	400	40.0	
	6	63.5	35.0	330	57.7	Jejunostomy.
						40 cc. per kg. of 1% NaCl subcutaneously.
	7	32.6	18.2	370	51.3	" " " " " 1 " " "
	8	30.0	16.8	490	41.9	" " " " " 1 " " "
	9	30.0	14.0	500	39.0	No injection.
	10	38.9	12.1	520	38.1	" "
	11	35.7	18.6	490	42.8	" "
	12	61.0	30.8	470	38.1	" "
	13	63.5	36.4	370	42.8	40 cc. per kg. of 1% NaCl subcutaneously.
	14	56.8	25.2	460	48.5	" " " " " 1 " " "
	15	34.1	16.8	540	45.7	No injection.
	16	48.2	24.5	500	38.1	" "
	17	115.0	51.1	460	44.7	40 cc. per kg. of 1% NaCl subcutaneously.
	18	66.3	35.7	520	39.0	No injection. Died.
22	0	26.5	13.2	510	27.5	Obstruction of jejunum.
	1	30.0	9.1	450	40.9	
	2	41.6	16.1	390	34.3	
	3	40.0	11.9	350	33.4	
	4	42.8	16.1	360	34.3	Jejunostomy.
						40 cc. per kg. of 1% NaCl subcutaneously.
	5	46.2	39.9	380	32.4	" " " " " 1 " " "
	6	30.6	8.4	430	38.1	" " " " " 1 " " "
	7	23.4	10.5	370	28.1	" " " " " 1 " " "
	8	24.0	8.4	550	36.2	No injection.
	9	28.2	15.4	530	37.2	" "
	10	45.0	26.6	500	36.2	" "
	11	41.0	21.1	440	46.6	40 cc. per kg. of 1% NaCl subcutaneously.
	12	30.0	9.8	480	42.8	" " " " " 1 " " "
	13	27.5	16.1	510	41.9	No injection. Died.
23	0	24.8	18.2	480	36.2	Obstruction of jejunum.
	1	31.6	7.7	480	39.0	
	2	25.9	10.5	450	34.3	

TABLE V—*Concluded.*

Dog No.	Day after operation.	Blood. Amount per 100 cc. Total non-protein nitrogen.	Urea nitrogen.	Chlorides.	CO_2-combining power.	Treatment.
		mg.	*mg.*	*mg.*	*vol. per cent*	
23	3	37.5	11.2	410	46.6	
	4	25.7	10.5	420	51.1	
	5	33.3	11.9	370	52.0	
	6	35.3	17.5	370	59.6	
	7	31.6	11.2	370	55.8	
	8	28.5	12.6	330	63.4	Jejunostomy.
						40 cc. per kg. of 1% NaCl subcutaneously.
	9	28.5	9.1	350	65.3	" " " " " 2 " " "
	10	23.8	9.1	430	54.9	" " " " " 1 " " "
	11	44.4	19.6	450	43.8	" " " " " 1 " " "
	12	34.5	16.8	470	48.3	" " " " " 1 " " "
	13	57.0	31.5	510	38.1	No injection. Died.
24	0	27.3	11.2	500	32.4	Obstruction of jejunum.
	1	28.5	6.3	440	41.9	
	2	31.6	9.8	420	46.6	
	3	29.4	12.6	400	48.3	
	4	27.7	9.8	390	48.3	
	5	32.6	7.0	390	39.0	
	6	30.3	14.0	330	51.1	Jejunostomy.
						40 cc. per kg. of 1% NaCl subcutaneously.
	7	32.6	9.1	390	48.3	" " " " " 2 " " "
	8	22.9	11.9	450	43.8	" " " " " 2 " " "
	9	20.2	7.0	490	43.8	" " " " " 1 " " "
	10	22.9	11.9	460	46.6	" " " " " 1 " " "
	11		18.6	480	42.8	" " " " " 1 " " "
	12	37.5	14.7	490	34.3	" " " " " 1 " " "
	13	60.5	33.6	500	40.9	No injection. Died.
25	0	31.6	10.5	490	37.2	Obstruction of jejunum.
	1	38.9	14.0	470	32.4	
	2	33.7	14.0	450	43.8	
	3	25.9	11.2	440	46.6	
	4	31.6	12.8	390	49.2	
	5	34.5	11.9	390	40.0	
	6	30.0	9.8	350	56.8	Jejunostomy.
						40 cc. per kg. of 1% NaCl subcutaneously.
	7	29.1	9.8	380	53.9	" " " " " 2 " " "
	8	28.5	9.8	460	33.4	" " " " " 1 " " "
	9	21.8	8.4	440	34.3	" " " " " 2 " " "
	10	33.7	9.1	580	33.4	No injection.
	11	30.0	7.7	540	39.0	" " Died.

but not beyond that of dogs having obstruction alone. The sodium chloride seems to be of value in prolonging life. The jejunostomy is apparently of no definite benefit.

Jejunostomy done after the development of a toxemia does not increase the duration of life nor does it alter the progress of the chemical changes in the blood.

In the last series, in which the jejunum was obstructed, followed later by jejunostomy and treatment with sodium chloride, life was definitely prolonged. Sodium chloride solution was given just before jejunostomy and thereafter in sufficient quantity to keep the blood chlorides within normal limits. It is difficult to explain why these animals live longer than those having jejunostomy and sodium chloride treatment from the beginning. The findings suggest that early jejunostomy not only did no good, but apparently did harm. Comparing the last series with the fourth series, it seems clear that the sodium chloride was of value in prolonging life.

CONCLUSIONS.

1. Jejunostomy does not prevent the development of the chemical changes of the blood characteristic of obstruction of the jejunum in the dog.

2. Jejunostomy following experimental obstruction of the jejunum has no beneficial effect upon the duration of life. There is some evidence that life may be shortened by early jejunostomy.

3. Treatment of jejunal obstruction with sodium chloride solution tends to prolong the life of animals regardless of jejunostomy.

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CALCIFICATION OF THE SUPRARENAL GLANDS OF CATS.

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PLATE 11.

(Received for publication, January 18, 1926.)

It is for the purpose of calling attention to a little known but apparently common disease of the suprarenal glands of cats that the following observations are published.

The only extensive paper on calcification of the suprarenal glands of cats is that of Brüschweiler (1) who reported finding 15 instances in 303 cats examined at Berne. He thought that the incidence of the disease increased with age and that it was probably dependent upon some disturbance of calcium metabolism secondary to chronic inflammations of the intestines. Kruse (2) in reporting an instance of bone formation in the suprarenal medulla of a monkey mentions that Wislocki showed him a calcified suprarenal of a cat.

During the past 5 years we have observed 64 cases of calcification of the suprarenals in 257 cats—approximately 25 per cent. Of the 257 cats, 6 were born and reared in the laboratory, but in none of these was calcification found. The remaining 251 were street cats whose average stay in the laboratory was less than 1 month. All of the 64 instances of calcification were, therefore, found in the street cats. As regards sex, 32 were females, 31 males, and in 1 the sex was not recorded. As regards age, we grouped them as follows: old adults, 7; adults, 21; young adults, 23; kittens, 13. While the ages are only approximate the groups are relatively accurate. Only those with milk teeth present were called kittens. Old adults included those with most of the incisors and canine teeth absent. Young adults included those with complete second sets of clean, white teeth, and adults those with one or more incisor teeth absent and more or less yellowish discoloration of those present.

We have seen this form of calcification only in cats. In a series of 328 dogs and approximately 2300 rabbits whose suprarenals were

examined microscopically, no cases were observed. Through the courtesy of Dr. C. H. Lenhart, I have examined his preparations of 150 pig, 200 sheep, and 125 cattle suprarenals obtained from the slaughter house without finding a case of this kind of calcification (calcification of the suprarenals in tuberculous and other local inflammatory conditions while very common in these animals has no relation to the present subject). A few cases of calcification of the suprarenals have been recorded in man. Those reported by Newsam (3) and Victor (4) may be referred to. Newsam's case, a child of 2 years and 7 months, apparently healthy, suddenly became unconscious and died in 14 hours. Autopsy showed calcification of the fascicular and reticular zones with true bone formation in the medulla. Victor's case was similar except that there was no ossification and the calcification was confined to the two inner layers of the cortex.

Clinical Manifestations.

The disease seems always associated with distemper. It is therefore more common in the young, and during the fall and spring months. Only a portion of the cats having distemper develop calcification, and in the cases of distemper ending fatally, it is often absent. It usually develops 2 to 3 weeks after the infection is manifest, and while recovery from distemper is usual, the calcification remains, and we have formed the impression that though extensive it may remain for years with the animal in good health. The condition may be suspected in its severe forms if the cat after 2 or 3 weeks of distemper fails to improve, loses weight, becomes weak and ataxic, the fur becomes rough, and it shows little desire for food—symptoms which resemble those seen in cats surviving double suprarenalectomy for 2 or 3 weeks.

Pathological Anatomy.

The most characteristic feature is the presence of areas of calcification in the fascicular zone (see Figs. 1 to 3). There are all degrees from the smallest areas, detectable only with the microscope, to almost complete calcification of the entire cortex. The process begins in the middle zone as small foci of degenerating cells at first without calcium deposition. Then as the lesion advances, calcium is deposited and in

the severest cases it extends through the glomerular and reticular layers. In the more advanced cases, it is readily detected on gross examination as smaller or larger pale yellowish areas on the surface of the gland. When the calcification is generalized, the gland may be fractured between the fingers in the same way as a calcified artery wall.

TABLE I.

Suprarenals with Calcification.

Cat No.	Weight of suprarenal (dried).	Calcium.		Remarks.
	mg.	*mg.*	*per cent*	
1	54.1	0.11	0.2	Very early lesions detected microscopically.
2	67.1	2.76	4.1	Advanced lesions detected microscopically.
3	73.0	3.81	5.2	" " " "
4	32.7	2.77	8.5	Very advanced lesions detected macroscopically.
5	92.2	8.68	9.4	" " " " "
				Suprarenals without Calcification.
6	49.8	0.01	0.02	
7	46.9	0.01	0.02	
8	55.2	0.03	0.05	
9	49.6	0.03	0.06	
10	23.8	0.05	0.08	
11	38.2			

Our series supports Brüschweiler's observation that it is always calcification and never ossification. We have seen very few cases where the medulla is at all involved and in these it was clearly secondary. In the early stages the calcification is soft and amorphous, while in the long standing and recovered cases the calcification is hard, crystalline, and usually such areas have a fibrous capsule. At no stage have we observed evidence of inflammatory exudate about the foci. In

the early stages, calcium is probably deposited in the form of soap, while in the later stages it changes to carbonate and phosphate. A few of the glands have been examined for calcium; the figures are presented in Table I.

The sequence of events leading to the deposition of calcium seems to be somewhat as follows: The toxin of distemper produces a specific degeneration of groups of cells in the fascicular layer, lime salts are deposited in these foci at first combined with fatty substances, later going over to carbonate and phosphate.

EXPERIMENTAL OBSERVATIONS.

We have not succeeded in producing the lesion. Sublethal injury and necrosis of the cortex by diphtheria toxin and by arsenic have been tried. In a large series of anesthetized rabbits and cats the cortex was frozen with ethyl chloride. Partial ligation of the blood vessels and trauma causing extensive hemorrhage into the cortex were tried. All of these procedures gave negative results. In some of the cats in which these experimental injuries were produced, calcification of the suprarenals was found at autopsy but the incidence was not greater than in those not so treated. Hence the calcification was probably present before the experimental injury (see Fig. 4). It seems, therefore, that the toxin producing the initial degeneration of the cells of the fascicular zone which leads to calcification is a highly specific one. It does not occur in association with ordinary bacterial infections as for example chronic wound infection or chronic pneumonia with empyema. Likewise it does not occur following the usual types of experimental injury as mentioned above.

SUMMARY.

Calcification of the fascicular zone of the cortex has been observed in 64 of 257 cats. It is always calcification and never ossification. It is more common in young animals and in our experience is associated with distemper. In its severe forms it may be recognized clinically. The symptoms resemble those seen in cats surviving double suprarenalectomy for 2 to 3 weeks. The toxin producing the focal degeneration is clearly a very specific one since attempts to produce such lesions by several types of experimental injury have failed.

The sequence of events appears to be similar to that present in other degenerative processes associated with calcification, namely cell injury and necrosis, deposition of calcium at first as fatty compounds which later change to carbonate and phosphate. It is suggested that this lesion should be considered in interpreting experiments in which cats are used.

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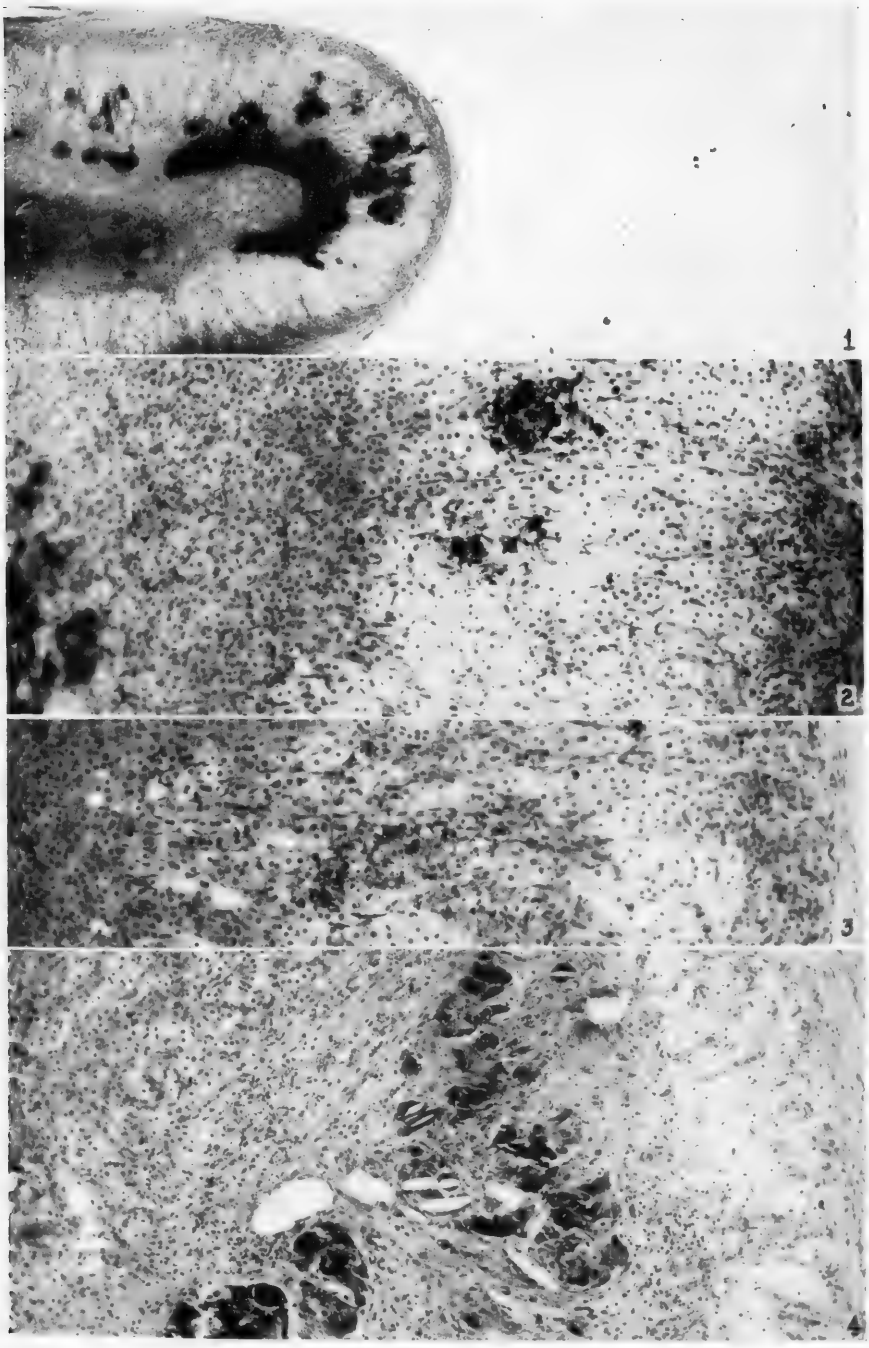
EXPLANATION OF PLATE 11.

FIG. 1. Illustrates the distribution of the calcification in relation to medulla and the cortical zones.

FIG. 2. Higher magnification of two small early foci.

FIG. 3. Very early lesion showing cell degeneration and beginning of calcification.

FIG. 4. Calcification in a gland frozen with ethyl chloride 25 days before removal. The degeneration and necrosis and cholesterol spaces are the result of freezing. The calcification was probably present at the time of freezing.



(Marine: Calcification of suprarenal glands of cats.)

UNIV. OF MICH.

SYPHILITIC MYOCARDITIS IN THE RABBIT.

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PLATES 12 TO 14.

(Received for publication, December 22, 1925.)

The disease produced in rabbits by local inoculation with highly virulent strains of *Treponema pallidum* bears a close resemblance to human syphilis in many respects and is distinctly different in others. The analogy is especially close during the early stages of the disease. As the infecting organisms multiply at the site of inoculation, a primary lesion develops and treponemata are distributed to all parts of the body by way of the blood vessels and lymphatics, giving rise to secondary lesions in such organs or tissues as the skin and mucous membranes, bones, and eyes; in male rabbits, an uninoculated testicle is almost invariably affected; and destructive lesions are frequently found in the lymph nodes draining an area that is the seat of a primary or secondary focus of infection. Furthermore, treponemata are present in the blood in variable numbers throughout the period of active primary and secondary infection and may be recovered from the lymph nodes and other organs at any time during the life of the animal.

We have, however, no definite knowledge of the occurrence of visceral syphilis in the rabbit comparable to the disease seen in man. A variety of lesions, gross and microscopic, are found in the internal organs of rabbits infected with *Treponema pallidum* and some of these have been attributed to syphilis; this is especially true of certain microscopic lesions in the central nervous system, but in no case has it been shown that any of these conditions were of syphilitic origin. The evidence submitted is largely morphological, reinforced in some cases by animal inoculation. The lesions described are, however, indistinguishable from those seen in the organs of normal rabbits, and the use of animal inoculation has added nothing to the morpho-

logical evidence since, at the time chosen for the inoculation of tissue emulsions, it is practically certain that the blood contained treponemata in sufficient numbers to render an emulsion of any organ infective. Under these circumstances, a positive result is of no value unless it is controlled by an accurate determination of the relative infectivity of the blood and tissue emulsion; even this would leave the matter in doubt as treponemata may be recovered from organs that show no characteristic lesions.

There is no doubt that the experimental infection differs from that of man with respect to the occurrence of visceral disease. Some of the lesions seen in the internal organs of rabbits may be due to syphilis, but the evidence available at the present time would indicate that, if visceral lesions occur at all, they are extremely rare or very minute, and the nature of the lesions, the frequency with which they occur, and the biological factors which account for the presence or absence of visceral lesions are still undetermined.

During the past 10 years we have examined a great many rabbits infected with *Treponema pallidum* and in a number of instances have found a granulomatous myocarditis which appears to be peculiar to syphilitic rabbits. The object of this paper is to report the results of a study of six animals that showed characteristic and pronounced macroscopic and microscopic lesions of the myocardium. The cases to be reported were observed during 1919, 1920, and 1921, but, as we were unable to obtain conclusive proof of their syphilitic origin at that time, publication was deferred with the hope that other cases might be encountered, and to afford an opportunity for a more exhaustive study of myocardial affections in rabbits that were not infected with *Treponema pallidum* since it appeared that the final decision of origin might rest upon a differentiation of the myocardial lesions found in syphilitic and non-syphilitic rabbits.

EXPERIMENTAL.

Clinical History and Postmortem Examination.

Rabbit 1.—Inoculated December 10, 1918, in both scrota with the Zinsser-Hopkins strain of *Treponema pallidum.* The primary lesions were small and of the type of a diffuse infiltration with extension along the perivascular lymphatics. There was an unusually marked lymphadenitis and in March, 1919, a small and

transient periosteal nodule developed on one of the bones of the forearm. On April 29, the animal was killed for postmortem examination. Duration of infection 140 days.

The pericardium was filled with a clear almost colorless fluid. The heart was considerably enlarged and in the wall of the left ventricle there was a conspicuous mass composed of a pale, semitranslucent tissue of a firm, elastic consistency. This mass occupied the greater part of the outer wall of the left ventricle, the lower half of the septum, and a small portion of the wall of the right ventricle in the region of the apex; it was clearly demarcated at the periphery.

Rabbit 2.—Inoculated February 24, 1919, in both scrota with the Nichols strain of *Treponema pallidum*. The primary lesions developed rapidly and on March 21 (25 days after inoculation) the animal was used in a therapeutic experiment. The chancres were completely healed at the end of 2 weeks but 2 weeks later an active orchitis developed in the right testicle; the left testicle also became affected and a small periosteal granuloma developed on the left external malleolus but there was no recurrence of the scrotal lesions. The right testicle was removed May 15[1] and the animal was killed for postmortem examination on May 22. Duration of infection 87 days; time from treatment 62 days, from appearance of testicular lesions 31 days.

The pericardium contained an excess of clear fluid. In the outer wall of the left ventricle there was a circumscribed nodule which presented essentially the same appearance as the lesion described in Rabbit 1 (Figs. 1 and 2). This mass protruded above the level of the surrounding muscle and measured 1.2 cm. in cross-diameter by 1.0 cm. in thickness. The lesion was wedge-shaped with the base toward the endocardium. The cut surface was moist and distinctly slimy.

Rabbit 3.—Inoculated in both testicles October 17, 1919, with a lymph node emulsion from an animal infected with the Nichols strain of *Treponema pallidum*. A bilateral orchitis developed after an unusually short incubation period for this type of inoculation (28 days). The right testicle was removed on November 19. During the following week the lesion in the left testicle increased at an extremely rapid rate, forming a mass about three times the size of the normal testicle. The animal was killed 39 days after inoculation or 11 days after the appearance of the initial lesion.

Externally, the heart presented a normal appearance but on opening the left ventricle a large part of the septum and a portion of the outer wall were found to contain a thin mass of pale, yellowish gray tissue (Fig. 3). The affected area measured approximately 1.0 cm. in cross-diameter, it was slightly elevated, firm, and elastic, and extended 2.0 to 3.0 mm. into the underlying muscle.

Rabbit 4.—Inoculated in both testicles, December 3, 1919, with blood from an animal infected with the Zinsser-Hopkins strain of *Treponema pallidum*. A bilateral orchitis developed after an incubation period of 40 days. No other lesions were noted clinically. On February 10, 1919, the animal struggled

[1] All surgical operations were done under ether anesthesia.

while being examined, collapsed, and died immediately. Duration of infection 62 days, or 22 days from the appearance of the primary lesions.

The heart was unusually large. Both ventricles and auricles were greatly dilated and filled with blood, giving the heart a distinct globoid shape which was not materially altered by *rigor mortis* (Fig. 4; *cf.* Fig. 1). Externally, there was a definite but rather diffuse area of pale yellowish gray discoloration which extended entirely across the surface of the left ventricle and faded diffusely into the surrounding tissues. On section, it was found that this condition extended through the wall of the heart in increasing degree, forming a broad band which completely girdled the base of the left ventricle on its inner surface. There were a few petechial hemorrhages beneath the endocardium and in the substance of the papillary muscles.

All of the internal organs were intensely congested.

Rabbit 5.—Inoculated in both testicles December 2, 1919, with blood from a rabbit infected with the Nichols strain of *Treponema pallidum.* A bilateral orchitis developed at the end of 43 days and both testicles were removed at this time. Between the 86th and 98th days after inoculation small secondary lesions developed on the nasal bones, eyelids, and the skin of the face. These were still present when the animal was killed for postmortem examination on March 22, 1920. Duration of infection 110 days; time from appearance of primary lesions and castration 67 days.

The heart was considerably enlarged and the pericardium slightly thickened. In the region of the apex there was a large, irregular, slightly depressed lesion in the wall of the left ventricle which presented the appearance of a fresh scar with lines of translucent tissue radiating outward into the surrounding muscle. The lesion extended through the wall of the ventricle, forming an irregular band which encircled the left ventricle in the region of the apex (Fig. 5). The left ventricle was dilated and its walls thickened; the mitral ring was widened; the left auricle was dilated and its wall thickened; the walls of the right ventricle were slightly thickened.

Rabbit 6.—Inoculated in the right testicle January 17, 1921, with the Zinsser-Hopkins strain of *Treponema pallidum.* The course of disease in this animal was rather severe. The incubation period was 8 days; metastatic lesions developed in the left testicle and tunics 30 days after inoculation and were followed by a rather extensive involvement of lesions of bones (64 days), mucocutaneous borders (87 days), and skin (93 days). Significant features of the disease were: a very short incubation period and the early development of lesions in the uninoculated testicle, the occurrence of slight testicular lesions followed by atrophy of the testicles and extension of the infection to the tunics and scrotum with the formation of numerous small nodules, the presence of longer intervals than usual between the development of successive groups of generalized lesions, the destructive character of the lesions, and a persistent activity of all lesions for a period of more than 6 months associated with distinct constitutional symptoms and pronounced physical deterioration. The lesions healed during the summer and the animal

was killed for postmortem examination January 26, 1922. Duration of infection 1 year.

The heart was considerably enlarged (weight 3.66 gm. per kilo as compared with a mean normal weight of 2.85 gm.). In the outer wall of the left ventricle there was a dense, opaque, yellowish white mass which measured about 1.0 cm. in diameter; it was slightly depressed and fairly well circumscribed. This mass extended through the wall of the ventricle and on the inner surface of the ventricle a series of irregular opaque white lines and patches radiated outward from the central lesion (Fig. 6). The mitral ring was widened and the walls of the left auricle and ventricle were thickened and the papillary muscles hypertrophied; there was slight thickening of the wall of the right ventricle.

Examination for Treponemata.

Fluid expressed from the myocardial lesions of Rabbits 2 and 3 was examined by dark-field illumination but no treponemata were found. Tissues from the lesions of Rabbits 1 to 4 were also stained by the Levaditi method but no organisms could be found in the sections or in sections of control tissues that were known to contain treponemata in abundance.

Microscopic Examination.

Microscopically, the hearts of all animals showed lesions of a common type. The fundamental change was a myocardial degeneration and necrosis associated with proliferation of connective tissue and cellular infiltration. The condition found in individual animals differed with respect to the apparent age and extent of the lesions and the details of the pathological process. The earliest lesions were in Rabbits 2, 3, and 4. That in No. 4 was probably the earliest of the group and will serve as the basis for a description of the conditions present at this stage in the development of the lesions.

The outstanding feature of the condition found in the heart of Rabbit 4 was a widespread degeneration and necrosis of individual muscle fibers. The change was most marked in the immediate vicinity of small and medium size blood vessels, as shown in Fig. 7, but was not confined to such locations. The majority of the muscle fibers (Fig. 8) were swollen, pale staining, coarsely granular, or vacuolated, and many of them were in process of disintegration; others were small and granular or showed an intense hyaline change. The

nuclei stained very irregularly. Some were swollen and extremely pale, others were shrunken, pycnotic, and irregular in outline.

The growth of connective tissue and infiltration of wandering cells were comparatively slight. The connective tissue was distinctly myxomatous in type and was practically confined to those areas in which the degeneration and necrosis of muscle fibers were most pronounced. A few lymphocytes, plasma cells, and polymorphonuclear leucocytes, chiefly eosinophils, were distributed diffusely through these same areas; they were present in about equal proportions and showed no tendency to perivascular arrangement other than that incident to the location of the lesions.

No definite alteration could be detected in the vessels as a whole. A few of the medium size vessels showed a suggestion of degeneration of the intima and media and occasionally leucocytes were seen in process of migration through the vessel wall.

The most pronounced lesions were in the papillary muscles and trabeculæ. Several of these structures showed extensive degeneration and necrosis of muscle elements with proliferation of connective tissue, cellular infiltration, and hemorrhage. The condition seen elsewhere was essentially the same as that shown in Figs. 7 and 8.

The lesions in Rabbits 2 and 3 appeared to be more advanced and of a milder grade. The degeneration and necrosis of muscle fibers were not so marked or extensive as in Rabbit 4 but there was a distinct interstitial edema or mucoid infiltration and the proliferation of connective tissue was greater; there was also a slight increase in the cellular infiltration (Figs. 9 and 10). The feature of especial interest, however, was a more definite perivascular localization of the myocardial lesions as shown in Fig. 9. In other places, the lesion still retained a diffuse character (Fig. 10). The only difference between Rabbits 2 and 3 was that the focal lesions were slightly larger in No. 3 while the diffuse reaction was less.

Rabbit 1 (Figs. 11 and 12) showed a condition which appeared to be an advanced stage of a lesion comparable to that seen in Rabbit 4. The degeneration and necrosis of muscle fibers were widespread and diffuse with definite intensification in the regions about the blood vessels. In this instance, there was considerable replacement of heart muscle by a myxomatous granulation tissue and a well marked cellular infiltration without definite perivascular arrangement.

The final stages of the lesion were seen in Rabbits 5 and 6 (Fig. 13). In these cases, there were dense masses of fibrous connective tissue which contained neither heart muscle nor wandering cells. These masses gradually diminished from the center toward the periphery, giving place to a finer network of slightly cellular connective tissue surrounding small islands and strands of heart muscle and separating individual muscle fibers. Some of the fibers were hypertrophied, others were atrophic, and many of them showed pronounced degenerative changes. In their grosser aspects, the healed lesions suggested a terminal stage of a process analogous to that seen in Figs. 11 and 12, while in their finer details they were more analogous to the condition shown in Fig. 10.

DISCUSSION AND CONCLUSIONS.

The only evidence that could be accepted as conclusive proof that the myocardial lesions reported were due to syphilis is the demonstration of treponemata in numbers by dark-field illumination or in stained preparations. Animal inoculation would have contributed little or nothing to the diagnosis as five of the six rabbits showed active syphilitic lesions in other parts of the body at the time of autopsy and it was practically certain that their blood contained living organisms. In Rabbit 6, it is possible that animal inoculation might have been helpful on the general principle that a tissue once the seat of a localized syphilitic infection is prone to harbor treponemata indefinitely.

At the time these lesions were noted, however, we were by no means certain that they were syphilitic and were inclined to trust to the demonstration of treponemata in tissues for a diagnosis. The demonstration of organisms by any method would have been decisive but the failure to do so has only a limited significance. The dark-field examinations were limited to fluid expressed from the cut surface of the lesions and this may not have been sufficient to dislodge organisms from the tissues. Still, if treponemata were present in these particular instances it would appear that they were not numerous. The failure to find them in tissues treated by the Levaditi method is of no particular significance as control tissues were also negative. In our experience, such results are not unusual, particularly so where

one is dealing with strains of *pallidum* that have been carried in rabbits for many years.

The interpretation of the conditions described rests, therefore, on the pathology of the lesions and the circumstances of their occurrence. The clinical history is strongly suggestive of a relation between the myocardial lesions and the syphilitic infection in five of the six rabbits. In these cases, the myocarditis developed at about the time other generalized manifestations of disease usually occur and in several instances was closely associated with the development of such lesions. In the sixth animal, the interval between the period of active disease and autopsy was too long to permit of definite correlation but, in this case, all lesions had healed.

Another connecting link is to be found in the striking macroscopic resemblance of the myocardial granulomata to circumscribed syphilitic lesions in any other organ or tissue of the rabbit that have not been exposed to secondary infection. Microscopically, the analogy is not complete in all respects. It is well to remember, however, that the histology of syphilitic lesions in any given organ or tissue varies within certain limits, as do the clinical manifestations of disease, that there are even greater differences between the lesions of different organs, and that there is no characteristic feature of the pathological process that is constantly present, unless it be some evidence of a relation of the primary injury to blood vessels and lymphatics. We might be misled, therefore, if we expected a syphilitic myocarditis to agree in all respects with syphilitic lesions in other tissues.

So far as the rabbit is concerned, we have no closely analogous condition with which a comparison might be made but the lesions under consideration agree in almost every detail with the description given by Warthin of syphilitic myocarditis in man (1, 2). The only essential difference is the absence of a perivascular accumulation of wandering cells, but even this difference is offset by a perivascular arrangement of the lesions themselves. Moreover, as far as the changes go, they are typically syphilitic. The features of the conventional syphilitic reaction that are absent, or present in relatively slight degree, are decided alterations in the blood vessels and a pronounced perivascular arrangement of wandering cells. Where these conditions are present they have a distinct diagnostic value but they

are not indispensable features of the syphilitic reaction in man or animals and are frequently slight or entirely absent in the critical or more malignant lesions of rabbits.

Myocardial lesions of various types are comparatively common in stock rabbits (3) but the great majority of them are of microscopic proportions and bear no resemblance to the conditions described above. In very rare instances, a suppurative myocarditis may lead to the formation of granulomatous masses or scars but even these are readily identified by the presence of mass necrosis, dense infiltrations of polymorphonuclear leucocytes, and miliary abscesses in the surrounding tissues as shown in Figs. 14 and 15.

As far as we have been able to determine, the lesions described are peculiar to syphilitic rabbits, and it seems practically certain that they are true examples of syphilitic myocarditis. Similar lesions might be produced by other experimental agencies but the histopathology is sufficient to distinguish them from any known lesion of spontaneous origin. The distinctive features of the condition are a primary degeneration and necrosis of individual muscle fibers associated with a growth of myxomatous connective tissue and infiltration of wandering cells with a predilection for tissues in the immediate vicinity of small and medium size blood vessels. The peculiar nature of the degenerative change in the muscle, the myxomatous character of the connective tissue, interstitial edema or mucoid infiltration, and the mixture of mononuclear and polymorphonuclear leucocytes with eosinophilic granules appear to be significant features of the active lesion. Healing is apparently accomplished by simple conversion of cellular connective tissue into dense fibrous tissue rather than by mass necrosis and softening with subsequent replacement of necrotic tissue as in the case of large gummata or of bacterial infections. Attention may also be called to the fact that the most extensive changes and probably the earliest lesions are found in close association with the endocardium. That is, the lesions of the heart occupy essentially the same position and structurally they are of the same character as the vascular lesions of syphilis in man or experimental animals. We regard this as a highly significant feature of the myocardial affection.

Very little can be said concerning the frequency with which lesions

of this type occur. We have limited our report to six cases in which there were conspicuous granulomatous masses in order to avoid any possible confusion with conditions that are of microscopic proportions. It is obvious, however, that smaller lesions must occur and we have found a number of cases in which a diagnosis could be made without difficulty on the microscopic findings. But, the material available cannot be used for an estimation of the probable frequency of myocardial involvement as the time of occurrence and the fate of small lesions are factors for which proper corrections cannot be made.

Still, the present studies have an important bearing on the question of myocardial involvement and the biological relation of syphilitic myocarditis to other manifestations of disease. The fact that so few cases of pronounced granulomatous myocarditis have been encountered is sufficient evidence that this form of the disease is rare and that its occurrence is probably determined by some unusual combination of conditions. In this connection, it is of interest to note that the cases reported occurred in the spring or fall of 3 successive years (1919–21) which coincides with a 3 year period during which syphilis was much more severe in our animals than at any other time during the 10 years that we have been studying this disease. Moreover, they occurred at times and in groups of animals in which syphilis displayed a maximum severity. Still, with a single exception (Rabbit 6), the disease in these particular animals did not appear to be severe or especially mild as compared with other animals in their respective groups. There was, however, some peculiar condition in each case such as a short incubation period, a deficient or unusually active primary reaction, an experimental interference with the course of disease, or a slowly progressive and persistent course of primary and secondary infection.

These circumstances, taken in conjunction with what is known concerning the evolution of syphilis in the rabbit (4, 5), provide an explanation for the occurrence of lesions that under ordinary circumstances do not occur at all or are of such minute proportions as to escape detection. Omitting the lymph nodes, the usual sequence of development of known syphilitic lesions in the rabbit, after testicular inoculation, is as follows: primary orchitis, metastatic orchitis or periorchitis, lesions of periosteum and bone, skin and mucous mem-

branes, and eyes. The sequence is slightly different in the case of cutaneous or subcutaneous inoculations. The course of disease may be spontaneously arrested at any point in its progress or whenever the immunity developed is sufficient to bring the infection under control.

We have no definite knowledge concerning visceral susceptibility in animals but it is apparent that the viscera in general are either less susceptible to syphilitic infection than the parts enumerated above, or under ordinary circumstances, they are more effectually protected by reactions in other tissues. The evidence available indicates that the factor of protection is the more important of the two but there has been some uncertainty as to whether the protection is conferred during the course of the early reactions or during the terminal stages of the disease. The evidence obtained on this point is conclusive. In the experimental animal, the sequence of involvement and of protection is the same and it can be definitely stated that in five of the six rabbits involvement of the myocardium occurred at or near the time of occurrence of the earliest generalized lesions. In Rabbit 3 the myocardial lesion developed within 11 days after the appearance of the initial lesion in the testicles and in No. 4 the animal died with acute cardiac dilatation 22 days after the appearance of the primary lesions. That is, the time of occurrence of the myocarditis coincided with the occurrence of the earliest cases of metastatic orchitis. In Rabbit 5, the indications are that a similar condition existed as the myocardial lesions healed while those in other tissues were still active. The time relations are not so clearly defined in the two other animals (Nos. 1 and 2) but we know by comparison that the myocarditis occurred early and either coincided with or antedated the development of lesions in the bones and skin.

From a consideration of the facts cited above, it appears that the occurrence of myocardial lesions in the rabbit is determined or prevented by conditions that affect the early reactions. Theoretically, therefore, such lesions might be expected to occur if the usual protection afforded by primary and metastatic lesions failed either on account of the severity of the disease or of some individual or experimentally induced deficiency. Both of these conditions are applicable in the present instance. At the time these cases of myocarditis occurred, the

average course of disease was especially severe; in one group of animals the course of events was interfered with by castration or treatment or by both, while in the other, for unknown reasons, the primary and metastatic lesions were comparatively slight. Apparently, the disease progressed so rapidly in one group of animals that there was no opportunity for the development of the usual protection, while in the other, the early reaction was inadequate.

From the biological point of view, it is of especial importance to note that involvement of the myocardium in the rabbit is an early rather than a late event in the evolution of disease. The same may be true of other forms of visceral syphilis. Most of the examinations that have been made with a view to discovering visceral lesions in rabbits have been carried out at comparatively late stages of the infection, and this may explain the apparent absence of visceral disease in the rabbit. With the information now available, it is not unlikely that systematic investigations, carried out at appropriate times and under favorable circumstances, may yield important information concerning visceral syphilis, and myocarditis in particular.

In conclusion, it should be pointed out that these observations cannot be applied to syphilis in man without making proper allowance for fundamental differences in the mechanism of the reaction to infection. They suggest, however, that myocardial involvement, in untreated cases of syphilis, is apt to occur early rather than late and that disturbance of the early reaction to infection, as by inadequate treatment, may increase the incidence and severity of myocardial infections which would become manifest at a later period. There is also conclusive evidence of the occurrence of a true syphilitic myocarditis which is not dependent upon an antecedent disease of the blood vessels but is of the same general order as the vascular lesions of syphilis. Biologically, this is what one would expect, as the heart is merely a specialized portion of the vascular system and should occupy a position comparable to that of the blood vessels with respect to the occurrence, distribution, and character of syphilitic lesions.

SUMMARY.

In the course of routine postmortem examinations of rabbits infected with *Treponema pallidum*, six cases of pronounced granuloma-

tous myocarditis were encountered. Treponemata were not demonstrated in the lesions, but the clinical history, and the gross and microscopic appearance of the lesions seemed to warrant a diagnosis of syphilitic myocarditis. The lesions measured 1.0 cm. or more in diameter and histologically were practically identical with those described by Warthin in cases of syphilitic myocarditis in man.

These are the first cases of syphilitic myocarditis or of visceral syphilis in the rabbit that have been reported.

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EXPLANATION OF PLATES.

PLATE 12.

FIGS. 1 and 2. Heart of Rabbit 2. In Fig. 2, the pale wedge-shaped area in the cut edge of the myocardium shows the relative extent of the involvement with respect to the endocardial and epicardial surfaces.

FIG. 3. Heart of Rabbit 3. The gross lesion in this animal was strictly endocardial and subendocardial.

FIG. 4. Heart of Rabbit 4. The feature of especial interest is the marked dilatation. The location of the specific lesion is not clearly defined as the entire wall of the left ventricle was more or less affected.

FIG. 5. Heart of Rabbit 5. The scarred area extends from a point near the apex on the right, entirely across the inner wall of the left ventricle. Note also the size of the ventricle and the breadth of the mitral ring.

FIG. 6. Heart of Rabbit 6. There is a prominent scar in the wall of the left ventricle; the walls of both the ventricle and the auricle show distinct hypertrophy.

FIG. 7. Rabbit 4. The darker hyaline fibers give a general idea of the microscopic distribution of the lesions in this animal with reference to one large and several small vessels. × 80.

FIG. 8. A portion of the area shown in Fig. 7. Note the extensive degeneration and necrosis of muscle fibers as compared with the proliferation of connective tissue and cellular infiltration. This is a very acute lesion. × 250.

PLATE 13.

FIG. 9. Rabbit 2. An early perivascular focus of myocarditis. × 250.

FIG. 10. An area of more diffuse myocarditis than that in Fig. 9. The muscle fibers are widely separated by a myxomatous connective tissue and mucoid material. × 250.

FIG. 11. Rabbit 1. An advanced case of myocarditis presenting all of the characteristic features of the early lesions with more extensive degeneration and necrosis of muscle fibers, proliferation of connective tissue, and cellular infiltration. The relation of these lesions to the blood vessels is still evident. × 135.

PLATE 14.

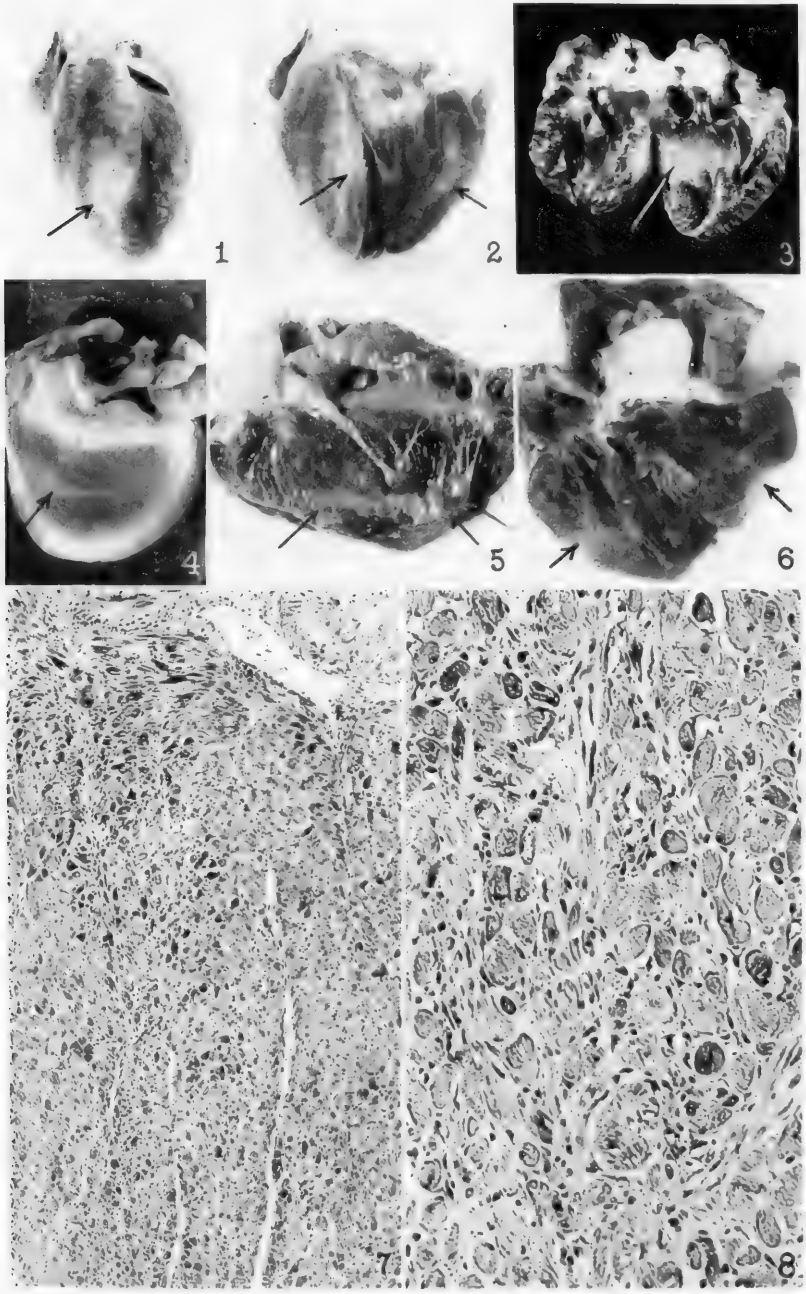
FIG. 12. A higher magnification of the lesion shown in Fig. 11. × 250.

FIG. 13. Rabbit 5. Healing myocarditis. × 80.

FIG. 14. Granulomatous myocarditis of bacterial origin (?) from the heart of an apparently normal rabbit. The greater part of the section shows granulation tissue with focal areas of necrosis infiltrated with polymorphonuclear leucocytes. The upper part of the section shows the dense polymorphonuclear infiltration of the adjacent myocardium. × 30.

FIG. 15. A miliary abscess in the myocardium near the lesion shown in Fig. 14. × 250.

Figs. 14 and 15 are to be compared with those showing lesions from syphilitic rabbits.



(Brown and Pearce: Syphilitic myocarditis in rabbit.)

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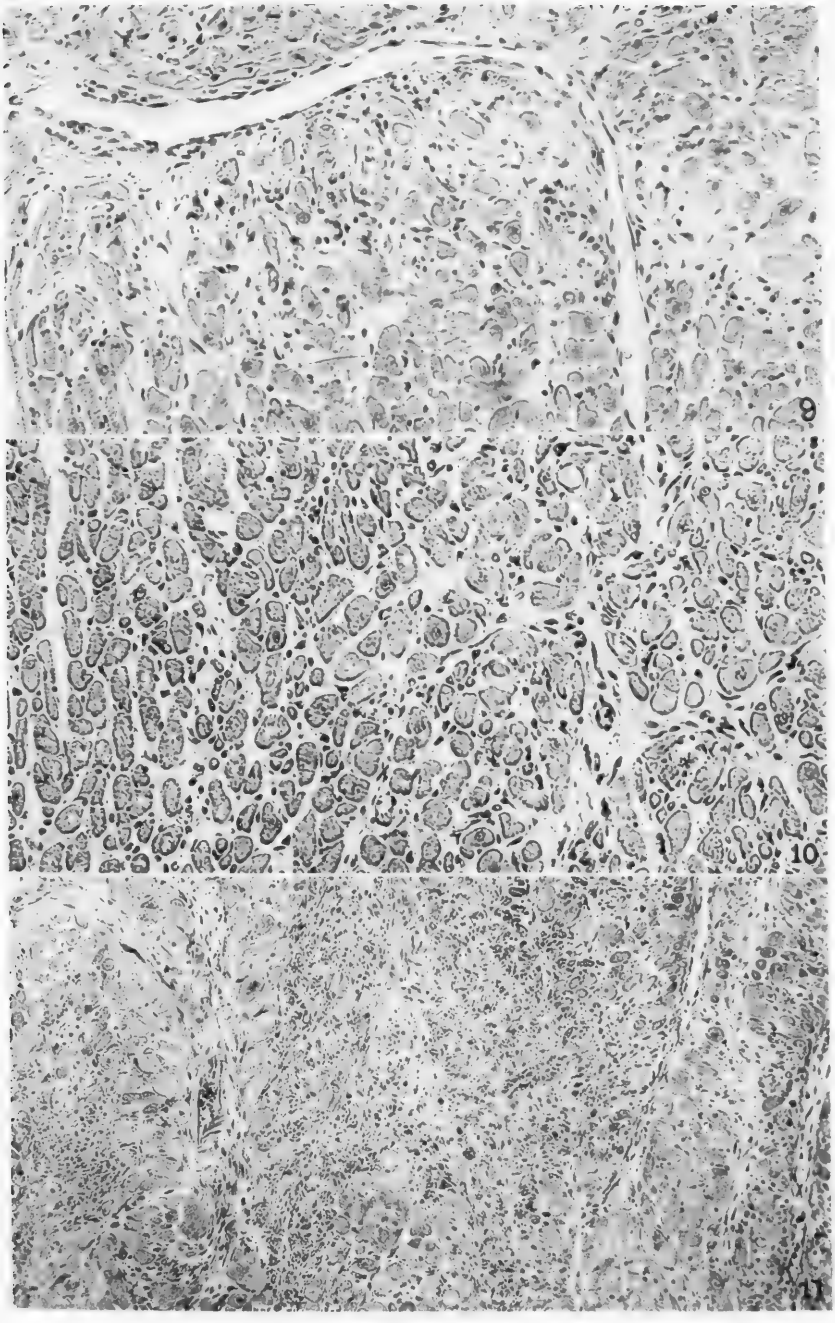

(Brown and Pearce: Syphilitic myocarditis in rabbit.)

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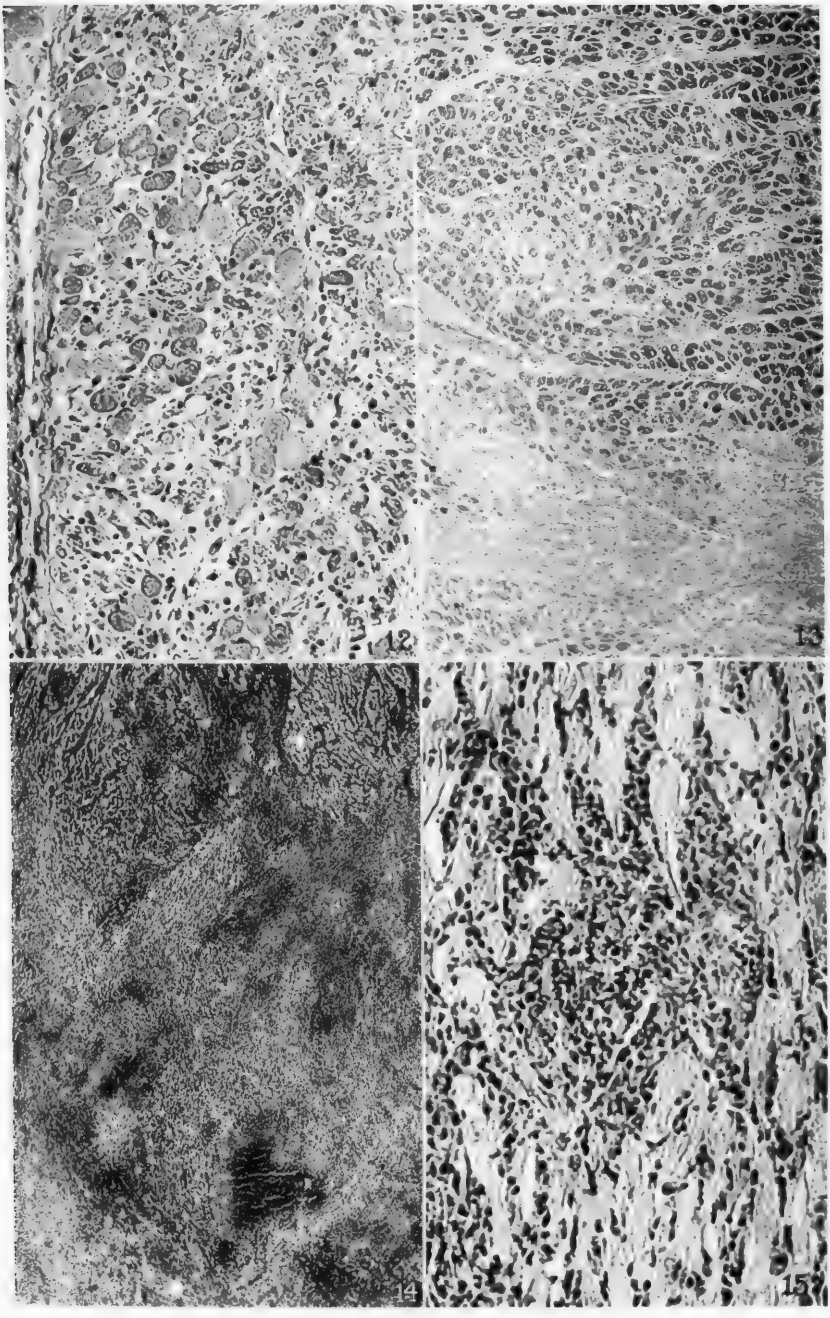

(Brown and Pearce: Syphilitic myocarditis in rabbit.)

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CULTIVATION OF RICKETTSIA-LIKE MICROORGANISMS FROM THE ROCKY MOUNTAIN SPOTTED FEVER TICK, DERMACENTOR ANDERSONI.

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PLATES 15 TO 18.

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A large number of microorganisms have been reported in recent years under the name of *Rickettsia*, or as *Rickettsia*-like microorganisms. The majority of these minute, for the most part intracellular, Gram-negative forms have been found in insects and arachnids.[1,2] Only in Rocky Mountain spotted fever,[3,4] typhus,[5] and heartwater discase[6] have pathogenic *Rickettsiæ* been definitely found in mammalian tissues.

Only a few of the *Rickettsiæ* have been isolated in pure culture. Nöller[7] reports successful cultivation of *Rickettsia melophagi*, Kuczynski[8] of *R. prowazeki*, and Sikora[9] of *R. pediculi*. By means of tissue culture technique, Wolbach and Schlesinger[10] obtained virulent cultures of *R. prowazeki* and *Dermacentroxenus rickettsi* from the tissues of experimentally infected animals.

Since the various *Rickettsiæ* thus far described are defined for the most part only by their host species and their morphological features, it is difficult to decide, in the lack of a comparison under controlled conditions, whether the forms found in different insects, notwithstanding their morphological similarity, are specific in each instance for the

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insect species, or whether the same form or group of forms infests several insects. Cultivation on artificial media would undoubtedly furnish more information with regard to their morphological and biological properties.

The present investigation has to do with the microorganisms of the spotted fever tick, *Dermacentor andersoni*, and represents the outcome of an attempt to cultivate the causative microorganism of spotted fever from infected ticks. A suspension of the viscera of an infected tick yielded on cultivation in a certain medium[11] a growth of minute, slightly motile, Gram-negative diplobacillary forms morphologically similar to the forms found in smears of the suspension; and 0.5 cc. of the culture (72 hours old) induced typical spotted fever, after 12 days, when intraperitoneally inoculated into the guinea pig. The fever lasted 6 days, and the animal showed the characteristic scrotal lesions of spotted fever. Transfers with blood taken at the height of fever induced typical spotted fever in other guinea pigs. Subcultures from the original culture, however, failed to induce either infection or immunity, as did also the original culture when 2 weeks old. Other immunological tests proved that the organism in question was in no way related to the virus of spotted fever, which had survived for a time in the original culture. The strain of spotted fever recovered from it was carried in guinea pigs for some time. The organism that had been cultivated resembles *Dermacentroxenus rickettsi* morphologically; it grows best at temperatures ranging from 15–26°C., and not at all at 37°C.

The incident showed that from the tissues of an infected tick a non-pathogenic microorganism closely resembling *Dermacentroxenus rickettsi* may be cultivated on special media. Through the generosity of Dr. R. R. Parker, of Hamilton, Montana, in keeping me supplied with adequate numbers of ticks, I have been able to carry out a systematic study of a large amount of material, both infective and non-infective.

[11] To 9 parts Hiss serum water containing various carbohydrates in series was added 1 part of 2 per cent nutrient agar. N/10 HCl was added to give a pH range of approximately 4.5 to 7.4.

Material and Mode of Investigation.

A number of ticks collected in the Bitter Root Valley, and termed "drag" ticks because of the method of collection, were fed on guinea pigs infected with a strain of spotted fever virus known as Barlow (which was given to me by Dr. R. R. Spencer, of Hamilton, Montana, in 1922) for 72 to 96 hours. Those which became engorged were kept at 12–15°C. during the period before use, which was never less than 7 days. Some drag ticks were fed on normal guinea pigs to determine the presence of natural infection, and in this way three new strains of spotted fever virus were obtained; these were termed Parker, Sawtooth, and Blodgett. The three guinea pigs which became infected from the naturally infected ticks were used for infecting other drag ticks. There were acquired, in this way therefore, four lots of drag ticks, each lot infected with a different strain of the virus.

Most of the ticks fed on normal guinea pigs did not induce infection, and these were regarded as non-infective ticks. The infectivity or non-infectivity of a given tick was determined in normal guinea pigs by feeding and by inoculation of emulsified viscera, or, when these procedures gave negative results, by immunity tests.

A tick which had become well engorged by feeding 72 to 96 hours was sterilized as far as possible on the outside with alcohol, ether, and sterile saline solution, and then cut into halves longitudinally with a sterile knife. One half was put into Regaud's fixative for sectioning and staining with Giemsa's and Gram-Weigert's stain. The other half was emulsified in a sterile mortar with 3 cc. of sterile saline solution. 1 cc. of the emulsion was injected intraperitoneally into a male guinea pig (450 to 500 gm.), and the remainder inoculated into various media. Smears were always made with the emulsion and sometimes also with the cut edge of the half tick before it was put into the fixative. This latter method often gave the best results. The smears were stained with Giemsa's and Gram's solutions.

All guinea pigs injected with the emulsions, unless they had typical spotted fever, were subsequently tested for immunity with blood from infected guinea pigs.

Cultural Methods.

In order to obtain comparable results, nearly equal numbers of infective and non-infective ticks were studied at the same time and under parallel conditions. Whether or not a given tick was infective was of course still unknown at the time when it was killed for cultivation purposes.

Several culture media were always used, and duplicate series were placed at 26°C. and 37°C. The special media used were: (1) leptospira medium (semisolid) plus carbohydrate (glucose, saccharose, maltose, mannose, levulose, mannitol, dextrin, xylose, inulin, salicin, dulcitol, and rhamnose) in a concentration of 0.2 per cent; (2) the same, containing a piece of fresh rabbit kidney; (3) the same as (1), but with an agar concentration of 1 per cent and slanted; (4) Novy and McNeal's medium containing the same sugars as the foregoing. Plain broth, agar slants, and yolk slants were always employed in addition to the special media. In the earlier experiments ordinary leptospira medium with and without fresh tissue was used, but it was soon omitted as less suitable than other media, notwithstanding the fact that *Leishmanias*, as well as leptospiras, grow vigorously at 15–26°C. on it, as they do also on media (1), (2), and (4). The quantity of tick suspension used for inoculation of the media was 0.1 cc.

As was to be expected, some of the emulsions contained more than one kind of microorganism. In such instances the growth obtained on semisolid medium was plated on the No. 4 medium to separate the organisms.

RESULTS.

Several varieties of bacteria have been isolated from emulsions representing 75 different ticks. The one most frequently encountered was very similar to that isolated in the preliminary experiment. Two others often grew on the special media, sometimes together. All three of the varieties cultivated have certain morphological features which simulate the pleomorphic phases of *Dermacentroxenus rickettsi* in spotted fever ticks, as described by previous investigators. For the sake of convenience, I shall designate the first type *Bacillus rickettsiformis*, the second *B. pseudoxerosis*, and the third *B. equidistans*.

Bacillus rickettsiformis, N. Sp.

Growth.—Initial cultures were obtained only on the special media, and at a temperature of 26°C., as minute, yellowish gray, semitransparent, shining, oval or round, slightly raised colonies near the condensation water. Consistency somewhat mucoid; after several days the colonies show a tendency to coalesce. The condensation water is slightly turbid and contains a grayish, rather sticky sediment. In a few instances a grayish surface growth was obtained on leptospira medium. Old strains grow well on broth, which shows slight opalescence, with surface pellicle and deposit, and on ordinary agar, on which some strains form a light yellowish pigment after several days. No growth is obtained under anaerobic conditions. One strain by gradual adaptation was made to grow at 37°C. Gelatin is not liquefied.

Morphology.—In young cultures the organisms are fairly uniform in size, measuring 0.3μ in width and 0.75μ in length; some of them are moderately motile. Single organisms are lanceolate, fusiform, or rod-shaped, and measure 0.25 to 0.35μ in width at the widest portion and 0.5 to 1μ in length, the longest about 1.4μ. Diplobacillary forms consist of two lanceolate forms joined end to end and measure about 2μ in length. The long rods do not occur in diploid form. In old cultures there is considerable pleomorphism, some individuals becoming oval, fusiform, or filamentous; the filamentous forms may show unevenness in width throughout the length of the body; some, especially those which settle down to the bottom of the fluid medium, have swollen ends. Vigorously growing individuals take a better stain than old ones. The organism is Gram-negative; it does not take Loeffler's methylene blue well. Staining with Giemsa's solution shows a light reddish violet body, of bipolar or diplobacillary shape, the middle portion being less distinctly stained. In bipolar elements the ends are abruptly pointed and show no stricture at the middle portion of the organism. Under certain conditions almost coccoid shapes may be assumed.

Strain Variations.—Variations in size exist among different strains, some being decidedly coarser or finer than the average type. In old surface colonies of some strains a light lemon-yellow color may appear.

Photomicrographs of the organisms found in smears and sections of infective and non-infective ticks, and of cultures of *B. rickettsiformis*, are shown for comparison (Figs. 1 to 23). Figs. 1 and 2 are dark-field views of one of the richest smears I have ever seen from non-infective ticks. The organisms are diploid and appear in paired refractile oval dots close together. In the center of Fig. 1 is a large round mass of organisms which look as if they were packed in a sac. This appearance is characteristic of rich emulsions.

When one compares carefully the forms found in the ticks and those assumed by *B. rickettsiformis* under various cultural conditions, one is tempted to regard them as identical. The causative agent of spotted fever, *Dermacentroxenus rickettsi*, is indistinguishable morphologically from the forms found in infective ticks, which again are similar to those found in non-infective ticks and those assumed by *B. rickettsiformis;* the only points of differentiation in the case of *Dermacentroxenus rickettsi* are its pathogenicity for certain mammalians and the fact that it is uncultivable by the methods employed in this investigation. Since ticks in general harbor in their tissues so called *Rickettsiæ*, the non-pathogenic tissue parasites in some of the wood ticks may likewise be regarded as *Rickettsia*, according to the present day usage

of the term, which is based on morphological characteristics. Should similar organisms be found in ticks collected in the eastern parts of the United States, we shall have an explanation of the presence, in non-infective ticks caught in spotted fever regions, of these forms which are morphologically so confusing when studied in connection with the spotted fever organism.

The foregoing morphological description applies to tick smears equally as well as to cultures of *B. rickettsiformis*. Some very minute (0.25 by 0.5μ) and some rather large (0.4 by 1μ) individuals occur in smears as well as in cultures, as do also coccoid forms (0.35 by 0.4μ).

In tick sections (salivary glands, etc.) single lanceolate individuals (0.25 to 0.35μ to 0.5 by 1μ) predominate; in some sections thinner forms are found, owing to different conditions of fixation and differentiation (Figs. 14, 19, 20). This appearance is also observed in cultures (Figs. 21, 22). Some coarser individuals are found in muscle (Fig. 18).

The organism of Rocky Mountain spotted fever in mammalian tissues presents striking resemblances to *B. rickettsiformis*, notwithstanding the fact that the latter is not related to it in any way. Smears show somewhat coarser organisms (Figs. 24 to 26) than sections (Figs. 27 to 29), owing to flattening of the former and greater decolorization of the latter in differentiation. Similar forms are seen in the smears of cultures of *B. rickettsiformis* (Figs. 30 to 34). Rather coarser individuals but with similar morphological characteristics are seen in Fig. 35.

Bacillus pseudoxerosis, N. Sp.

Growth.—Initial growth was obtained on the special media (slanted and semisolid) at 26°C. The colonies are light yellowish gray, shining, mucoid, and confluent. Subcultures grow fairly well on plain agar and broth, producing in the latter slight turbidity, with surface scum and stringy sediment. Optimum temperature for growth, 26°C. No growth under anaerobic conditions. Gelatin not liquefied.

Morphology.—Non-motile, slender, pleomorphic bacilli, measuring, in young surface cultures, 0.3 to 0.5μ in width and 0.4 to 2μ in length (Fig. 36). There is some resemblance to *B. xerosis* in arrangement and appearance. In somewhat older cultures groups of short rods are mingled with longer, often considerably thicker, rather curved individuals, 0.5 to 0.55μ by 3 to 4μ (Figs. 37, 38). The organism is somewhat resistant to Gram's stain. Bifurcated forms are seen in some cultures (Fig. 38).

Bacillus equidistans, N. Sp.

Growth.—Initial growth was obtained on the special slanted and semisolid media at 26°C. Light grayish white, somewhat fluorescent, round or oval, shining colonies on the surface of slants, tending to become confluent within several days. Growth of later subcultures obtained on plain broth and agar slants. The broth becomes moderately turbid, and after several days there is a heavy sticky scum and sediment. Colonies on slants are at first delicate but in a few days become heavier, mucoid, and have a light, brownish yellow tint. The organism grows best at 15–26°C.; 37°C. is less favorable for growth. No growth occurs under anaerobic conditions. Gelatin is not liquefied.

Morphology.—The most characteristic feature of this organism is its appearance of uniform dispersion, as if the individuals were purposely placed equidistant from one another. No motility has been observed. In young surface cultures there are many single individuals measuring 0.25 to 0.3μ by 0.4 to 0.8μ (Fig. 39). Diploid forms are sometimes predominant (Fig. 40), and there is an appearance of a narrow bridge between the two cells. In some instances minute coccoid forms, not unlike *Bacterium tularense*, may be assumed. This organism is negative by Gram's stain and stains reddish violet with Giemsa's solution. It appears to have a capsule, as indicated by a clear unstained or lightly stained zone several times wider than the cell itself. In older cultures are found irregularly curved filamentous forms, with a tendency to branch (Fig. 41).

The feces of ticks yielded the same three types of organisms on cultivation, *B. rickettsiformis* being most frequently present. It may be mentioned that the injections of suspensions of feces from twenty ticks which were infective (as shown by production of spotted fever by feeding on guinea pigs) produced some local infiltration and rise of temperature within 48 hours, but no spotted fever symptoms nor any immunity to the disease. This experiment merely shows the infrequency of spotted fever organisms in the feces of infected ticks and does not exclude the possibility of their occasional presence.

The results of fermentation tests with the three varieties of microorganisms cultivated are recorded in Table I. The medium used was Hiss's litmus serum water, and the cultures were kept 7 days at 26°C.

The strains of *B. rickettsiformis* used in this test only slightly attacked dextrose, galactose, levulose, and lactose. In some other experiments, however, strains were encountered which caused slight acidity with dextrose, maltose, xylose, and dextrin. *B. pseudoxerosis* affected the majority of carbohydrates employed but never sufficiently to cause any coagulation. *B. equidistans* fermented dextrose suffi-

ciently to produce marked acidity and coagulation without evolution of gas. Other carbohydrates were either very slightly or not at all fermented. In this respect the organism, though perhaps belonging to the group of *B. mucosus capsulatus*, appears to differ from any of the group so far known.

TABLE I.

Fermentation Reactions with the Microorganisms Cultivated from Ticks.

	B. rickettsiformis.		*B. pseudoxerosis.*		*B. equidistans.*	
	7 days.	30 days.	7 days.	30 days.	7 days.	30 days.
Dextrose.....	Trace acid.	+	Distinctly acid.	+++	Coagulation; acid; no gas.	+++
Saccharose....	−	−	" "	+++	−	−
Galactose.....	Trace acid.	+	" "	−	Slightly acid.	++
Maltose.....	−	−	" "	+++	" "	+++
Levulose.....	Trace acid.	+	" "	+++	" "	++
Xylose.....	−	−	" "	+	−	−
Lactose.....	Trace acid.	+	" "	++	Decolorized; no acid.	−
Mannose.....	−	−	" "	+++	Slightly acid.	++
Mannitol.....	−	−	" "	+++	−	−
Dulcitol.....	−	−	−		−	−
Raffinose.....	−	−	−		−	−
Rhamnose....	±	+	Trace acid.	+++	−	−
Dextrin.....	−	−	" "	+++	Markedly acid; no gas.	+++
Inulin.....	−	−	" "	+++	Trace acid.	−
Salicin.....	−	−	" "	+	−	−

Frequency and Distribution of the Various Organisms among Wood Ticks.

Most of the tick emulsions were sterile as tested with plain media (agar and broth) and with special media at 37°C., but the majority yielded some colonies on the special media after several days incubation at 26°C. Subsequent purification by plating showed that most of the ticks contained *B. rickettsiformis*, some *B. pseudoxerosis* or *B. equidistans* in addition, while a few yielded only one of the latter. In Tables II to VI are recorded the data regarding the findings in the case of each tick, including the results of biting tests, injection of

emulsion of viscera, and subsequent tests for specific immunity against spotted fever, the presence or absence of microorganisms in smears and sections, and finally the varieties of bacteria cultivated.

TABLE II.

Drag Ticks Which Did Not Induce Spotted Fever When Tested on Normal Guinea Pigs 12 Days Previously.

Tick No.	Results of					Bacteria isolated in culture.
	Feeding.	Inoculation of emulsion.	Immunity test.	Smears.	Sections.	
1	−	−	−	−	−	−
2	−	−	−	<+	+	*B. rickettsiformis.*
3	−	−	−	−	−	−
4	−	−	−	−	−	−
5	−	−	−	<+	+	*B. rickettsiformis.*
6	−	−	−	−	−	−
7	−	−	−	+	+++	*B. rickettsiformis.*
8	−	−	−	−	−	−
9	−	−	−	−	<+	*B. rickettsiformis; B. pseudoxerosis.*
10	−	−	−	−	−	−
11	−	−	−	−	−	−
12	−	−	−	−	−	−
13	−	−	−	−	−	−
14	−	−	−	−	−	−
15	−	−	−	+	++	*B. pseudoxerosis.*
16	−	−	−	+	++	" "
17	−	−	−	−	−	−
18	−	−	−	+++	++++	*B. pseudoxerosis.*
19	−	−	−	++	++++	" *rickettsiformis.*
20	−	−	−	−	−	−
21	−	−	−	−?	++	*B. rickettsiformis.*
22	−	−	−	−	−	−
23	−	−	−	−	−	−
24	−	−	−	−	−	−
25	−	−	−	+	++++	*B. rickettsiformis.*

Analysis of Results Shown in Tables II to VI.

Of 25 ticks fed previously on normal guinea pigs, none conferred either infection (as tested by biting experiments or inoculation of emulsified viscera) or immunity; that is, they were non-infective under the present experimental conditions. 15 of these yielded no culture,

5 yielded strains of *B. rickettsiformis*, 3 *B. pseudoxerosis*, and 2 *B. rickettsiformis* and *B. pseudoxerosis*.

Of 49 ticks fed previously on spotted fever guinea pigs, 14 conferred neither infection nor immunity; of these 2 yielded no cultures, 5 yielded *B. rickettsiformis*, 2 *B. pseudoxerosis*, 2 both of these organisms, 1 *B. equidistans*, 1 *B. pseudoxerosis* and *B. equidistans*, and 1 an undetermined Gram-negative bacillus.

TABLE III.

Drag Ticks Which Became Engorged When Fed on Spotted Fever Guinea Pigs (Barlow Strain) 12 Days Previously.

Tick No.	Results of					Bacteria isolated in culture.
	Feeding.	Inoculation of emulsion.	Immunity test.	Smears.	Sections.	
1	+	+		<+	+	*B. rickettsiformis.*
2	+	+		<+	+	" "
3	+	+		<+	+	−
4	+	+		+	++	−
5	+	+		+	+	−
6	+	+		+	++	*B. rickettsiformis.*
7	+	+		+	++	" "
8	+	+		±	+	" "
9	+	+		±	+	" "
10	−	−	+	+	+	" "
11	+	+		++	+++	" "
12	−	+		<+	++++	" "
13	+	+		<+	<+	" " *B. pseudoxerosis.*
14	+	+		++	+++	*B. rickettsiformis.*
15	−	+		+++	+++	" *pseudoxerosis.*
16	−	+		++++	+++	" *rickettsiformis.*
17	+	+		+++	+++	" "

Of the remaining 35 ticks, 16 (Group 1) caused typical spotted fever, both by biting and inoculation of emulsions, 13 (Group 2) failed to infect by biting, but the inoculation of emulsions conferred infection, 4 (Group 3) caused slight fever which proved by subsequent immunity test to have been due to spotted fever infection, and 2 (Group 4) were negative, but the animals which received the emulsified viscera proved to be immune to spotted fever; that is, all of these 35 ticks

carried the virus of spotted fever, 16 of them in highly virulent form. 10 of the ticks of Group 1 yielded *B. rickettsiformis* in culture, 2 *B. rickettsiformis* and *B. pseudoxerosis*, 1 *B. equidistans*, and 3 no growth. Of Group 2, 1 was sterile, and 12 yielded cultures, 4 *B. rickettsiformis*, 2 *B. pseudoxerosis*, 2 both, 3 *B. rickettsiformis* and B. *equidistans*, and 1 *B. equidistans*. Of Group 3, 1 was negative and 3 positive, 1 yielding *B. rickettsiformis*, 2 *B. pseudoxerosis*. One tick of Group 4 yielded *rickettsiformis* the other *rickettsiformis* and *pseudoxerosis*.

TABLE IV.

Drag Ticks Which Became Engorged When Fed on Spotted Fever Guinea Pigs (Parker Strain) 12 Days Previously.

Tick No.	Results of					Bacteria isolated in culture.
	Feeding.	Inoculation of emulsion.	Immunity test.	Smears.	Sections.	
1	−	−	−	++	+++	*B. rickettsiformis; B. pseudoxerosis.*
2	−	−	−	−	−	−
3	−	−	−	−	−	−
4	−	+	+	+	++++	*B. rickettsiformis; B. pseudoxerosis.*
5	−	+		+	+++	*B. rickettsiformis.*
6	−	−	−	+	++	" "
7	−	+	+	<+	<+	" "
8	−	−	−	−	−	−
9	+	+		++++	+++	*B. rickettsiformis; B. pseudoxerosis.*
10	+	+	+	++++	++++	*B. rickettsiformis.*
11	−	+	+	<+	+++	" " *B. equidistans.*

The percentage of non-infective ticks which yielded cultures was 40 per cent, of infective ticks 85 per cent. *B. rickettsiformis* occurred alone in 27 of the 52 ticks from which cultures were obtained (51 per cent), and in association with one of the organisms in 11, the total incidence of this organism being 73 per cent. *B. pseudoxerosis* occurred alone in 9 instances, and in association with one of the others in 9, the total incidence being 33 per cent, while *B. equidistans* occurred alone in 3 and with one of the others in 4 instances, the total incidence being 13 per cent.

TABLE V.

Drag Ticks Which Became Engorged When Fed on Spotted Fever Guinea Pigs (Sawtooth Strain) 12 Days Previously.

Tick No.	Results of					Bacteria isolated in culture.
	Feeding.	Inoculation of emulsion.	Immunity test.	Smears.	Sections.	
1	−	+	+	+++	+++	*B. pseudoxerosis.*
2	−	−	−	+	+	" "
3	−	−	−	±	<+	" *rickettsiformis.*
4	−	+	+	±	<+	" " *B. pseudoxerosis.*
5	−	+		<+	+	" *equidistans.*
6	−	−	+	−	+	" *rickettsiformis; B. pseudoxerosis.*
7	−	Fever.	+	−	+	" *pseudoxerosis.*
8	−	−	−	++	++++	" " *B. equidistans.*
9	−	+	+	+	+	*B. rickettsiformis; B. equidistans.*
10	−	+		++	+++++	*B. rickettsiformis; B. equidistans.*
11	−	Fever.	+	+++	+++++	*B. rickettsiformis.*

TABLE VI.

Drag Ticks Which Became Engorged When Fed on Spotted Fever Guinea Pigs (Blodgett Strain) 12 Days Previously.

Tick No.	Results of					Bacteria isolated in culture.
	Feeding.	Inoculation of emulsion.	Immunity test.	Smears.	Sections.	
1	−	−	−	<<+	+	*B. rickettsiformis; B. pseudoxerosis.*
2	−	−	−	±	+	*B. pseudoxerosis.*
3	−	−	−	±	+	" *rickettsiformis.*
4	−	−	−	<+	+++	Minute, motile, hemolytic, Gram-negative bacillus.
5	−	−	−	<+	+++	*B. equidistans.*
6	−	−	−	?	+	" *rickettsiformis.*
7	−	−	−	?	+	" "
8	−	Fever.	+	?	+	" *pseudoxerosis.*
9	−	"	+	+	+	−
10	+	+	+	++	++	*B. equidistans.*
11	−	+	+	±	+	−

Comparison of the cultural findings with the microscopic examination of the smears and sections shows that 17 ticks which conferred neither spotted fever nor immunity to the infection were negative both microscopically and by culture. All those ticks which yielded cultures of one organism or the other were positive also by microscopic examination, containing minute Gram-negative organisms in varying numbers in smears and sections, in some instances in enormous numbers. On the other hand, in 5 instances in which the ticks were definitely infective, the smears and sections showed the familiar Gram-negative organisms, yet no cultures were obtained from the emulsions. These forms may have been the true spotted fever organism, *Dermacentroxenus rickettsi*, and, like similar organisms in tissues from infected animals, could not be cultivated by the methods employed in the present investigation. The most striking point, however, is the morphological and tinctorial resemblance between the non-pathogenic organisms and the spotted fever parasites.

The source of the non-pathogenic microorganisms is unknown. I have never encountered any of them in mammalian bacterial flora, and they do not grow at body temperature. The optimum temperature of 12–26°C. or lower clearly indicates their adaptation to insects. For comparison, 5 drag ticks which had never been fed on guinea pigs were studied culturally. One of these contained *B. rickettsiformis*, the others were sterile. The result shows, however, that *B. rickettsiformis* does occur under natural conditions in the wood tick. The high incidence of these non-pathogenic parasites in the wood ticks used for the present study may not indicate their presence in wood ticks in general, for ticks collected in different localities and at different periods may not yield the same results.

Pathogenicity and Immunological Properties of the Bacterial Strains.

Guinea pigs, rabbits, and monkeys (*Macacus rhesus*) were inoculated with young cultures of *B. rickettsiformis*, *B. pseudoxerosis*, and *B. equidistans* grown on serum-containing media. In no instance were any lesions of spotted fever induced. There were a number of instances in which a continuous high temperature developed and lasted 2 to 5 days, but the spleen was usually normal, and most of the

animals recovered within a week. They were later tested for their susceptibility to the spotted fever virus, but in only two instances among 69 animals was any immunity present. In the two instances in which the animals became resistant to spotted fever, no significance was attached to the observation, because other animals which had received the same culture developed typical spotted fever.

Several rabbits were immunized with *B. rickettsiformis*, *B. pseudoxerosis*, and *B. equidistans*, respectively, by intravenous injection of cultures, and the immune serums were tested for neutralizing properties for the spotted fever virus. The results were negative. The action of spotted fever immune serums from hyperimmunized rabbits[12] and from recovered human beings[13] was tested *in vitro* upon each of the three organisms, but no specific agglutination or complement fixation was demonstrated. There was a tendency to the formation of clumps in some instances with human or rabbit serums (including normal ones) upon 24 to 48 hours standing at room temperature, owing to slow multiplication of the organisms.

The three organisms were also tested for agglutination and complement fixation with anti-*tularense* immune serum kindly furnished by Dr. Edward Francis, but the results were negative.

With regard to the use of tick emulsions as antigens in specific complement fixation tests with immune serums prepared in rabbits by repeated injections of virulent blood from infected guinea pigs, it was found that ticks fed on guinea pigs 7 days previous to the test still contain enough guinea pig blood to give strong fixation; of 20 ticks fed 40 days previously, fixation was still complete with the emulsions of 3 (females). The immune serum used for this work was of a titre such that 0.00001 cc. of guinea pig serum still completely fixed complement. To avoid this difficulty, human and guinea pig convalescent serums were tested with infective and non-infective tick emulsions. The results were uniformly negative, except in the case of two human (one normal, one convalescent) and two rabbit (one normal, one immune) serums, which fixed complement.

[12] Noguchi, H., *J. Exp. Med.*, 1923, xxxvii, 383.

[13] The specimens of serum from persons recovered from Rocky Mountain spotted fever were sent me through the kindness of Dr. W. F. Cogswell and Dr. John X. Newman, of the State Board of Health of Montana.

SUMMARY.

A systematic study of 74 ticks, the infectivity or non-infectivity of which was determined by biting experiments, inoculation of emulsions, and specific immunity tests, showed the presence in some instances of several types of microorganisms morphologically resembling the inciting microorganism of spotted fever. The most frequently isolated was *B. rickettsiformis, n. sp.*, those less commonly encountered were *B. pseudoxerosis, n. sp.*, and *B. equidistans, n. sp.* These organisms are non-pathogenic for the guinea pig, rabbit, and *Macacus rhesus*. In morphological features they resemble the forms found in smears and sections of the ticks, yet their presence had no relation to infectivity. Immunologically they are not related to the spotted fever virus. All three are pleomorphic under cultural conditions, and the question arises whether or not the minute non-pathogenic *Rickettsia* forms and the somewhat coarser symbionts found in *Dermacentor andersoni* are morphological variations due to variations in nutrition, oxygen tension, tissue reactions, etc., in the different tissues and cells in which the organisms are embedded. At all events, the differentiation of the non-pathogenic *Rickettsia*-like organisms from *Dermacentroxenus rickettsi* is extremely difficult.[14] In definitely infective ticks of the present study intranuclear forms were not constant.

A point of special interest is that these non-pathogenic microorganisms from ticks grow best at room temperature, in this respect resembling culturally certain flagellates inhabiting the alimentary tract of insects.[15] The difficulty of obtaining initial growth on artificial media and the gradual adaptation to less specialized media are other notable characteristics of these organisms. The possibility that *B. rickettsiformis* is a non-pathogenic phase of the spotted fever organism, comparable with the avirulent flagellate culture forms of

[14] Dr. E. V. Cowdry has been kind enough to look through the preparations described in this report without being informed whether they came from infective or non-infective ticks, in order that he might give an objective opinion concerning them. The sections of non-infective ticks contained forms which Dr. Cowdry regarded as *Dermacentroxenus rickettsi;* that is, forms indistinguishable from those found in sections of infected ticks. The organisms occurred in the salivary glands in both infective and non-infective ticks.

[15] Experiments to be reported upon later.

Leishmanias, seems remote in view of the negative immunological findings.

Hereditary transmission of *B. rickettsiformis* is clearly indicated by its presence in large numbers in ovaries and egg cells, a characteristic also of the spotted fever organism[16] and of other insect-borne *Rickettsiæ*.[1,2]

EXPLANATION OF PLATES.

PLATE 15.

FIGS. 1 and 2. Dark-field views of an impression film preparation from a non-infective tick. Film fixed in methyl alcohol, stained with Giemsa's solution, and photographed by dark-field illumination. Note the large unresolved mass of organisms in the left half of Fig. 1. × 900.

FIG. 3. Dark-field view of *B. rickettsiformis* cultivated for 6 days at 26°C. on Hiss serum water containing 1 per cent salicin. Note several agglomerates of the organism from which some individuals have become partly dissociated. × 1000.

FIG. 4. Same film preparation as Figs. 1 and 2, photographed in the ordinary way. × 1000.

FIG. 5. Smear preparation of an infective tick emulsion, fixed with buffered formalin. Gram (negative) counterstained with saturated alcoholic solution of fuchsin. × 1000.

FIG. 6. Smear preparation of an infective tick emulsion, stained in the same way as Fig. 5. × 1000.

FIG. 7. A cell from the hypoderm of an infected tick. Zenker's fixation followed by Giemsa's stain. Note the numerous rod-shaped organisms filling the cytoplasm of the cell. × 1000.

FIG. 8. Film preparation of the brain of an infected tick. Giemsa's stain. × 1000.

FIG. 9. Culture of *B. rickettsiformis* grown on ascites agar slant for 6 days at 26°C. Giemsa's stain. × 1000. Note the striking resemblance to the forms shown in Fig. 4.

FIG. 10. Culture of *B. rickettsiformis*. Blood slant 8 days old (26°C.). Giemsa's stain. × 1000.

FIG. 11. A young culture of *B. rickettsiformis* on blood sugar slant 48 hours old (26°C.). Giemsa's stain. × 1000.

FIG. 12. *B. rickettsiformis* on Nöller's medium (slant) grown 48 hours at 26°C. Giemsa's stain. × 1000. The forms shown in Figs. 11 and 12 bear a remarkable resemblance to those described by Wolbach (Fig. 34, Plate VII, *J. Med. Research*, 1919, xli, 1–197).

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[16] Ricketts, H. T., *J. Am. Med. Assn.*, 1907, xlix, 1278.

PLATE 16.

Magnification × 1000. Giemsa's stain.

FIGS. 13 and 14. *Rickettsia*-like organisms in the cells of the salivary gland of a non-infective tick. Regaud's fixation.

FIGS. 15 and 16. Similar organisms in the salivary ducts of a non-infective tick. Regaud's fixation.

FIG. 17. A cell of the hypoderm of a non-infective tick. Regaud's fixation.

FIG. 18. Similar organisms in the muscle fibres of a non-infective tick. Regaud's fixation.

FIG. 19. *Rickettsia*-like organisms in the epithelial wall of the rectal sac of an infective tick.

FIG. 20. Similar organisms in the brain of an infective tick (*Dermacentroxenus rickettsi?*). While these forms are decidedly more minute than some of the organisms found in sections of non-infective ticks, yet the minute forms shown in Fig. 14 (non-infective tick) are not morphologically and tinctorially distinguishable from those in the sections of infective ticks.

FIG. 21. *B. rickettsiformis* grown on slant agar for 8 days at 26°C.

FIG. 22. Same organism grown on serum agar slant for 8 days at 26°C.

FIG. 23. Same organism grown on Carrel's tissue culture medium for 3 days at 37°C.

All the forms found in the sections of ticks are to be observed also in these cultures.

PLATE 17.

Magnification × 1000. Giemsa's stain (except Fig. 31).

FIG. 24. Impression smear of the spleen of a guinea pig experimentally infected with the spotted fever virus. Buffered formalin fixation.

FIG. 25. Film preparation of teased lesions of tunica of a guinea pig infected with spotted fever. Buffered formalin fixation.

FIG. 26. Impression smear of the ear lesion of a guinea pig infected with spotted fever, showing an unusually large clump of spotted fever organisms in characteristic arrangement.

FIGS. 27 to 29. Spotted fever organisms in the endothelial cells of the small blood vessels at the sites of spotted fever lesions in guinea pigs. Regaud's fixation.

FIG. 30. *B. rickettsiformis* grown on ascites agar slant for 5 days at 37°C. (after gradual adaptation to this temperature).

FIGS. 31 and 32. Same organism grown 4 days at 37°C. on blood sugar slant. Fig. 31 Gram's stain (negative) counterstained with fuchsin; Fig. 32 Giemsa's stain.

FIG. 33. Same organism grown in serum broth at 37°C. for 6 days.

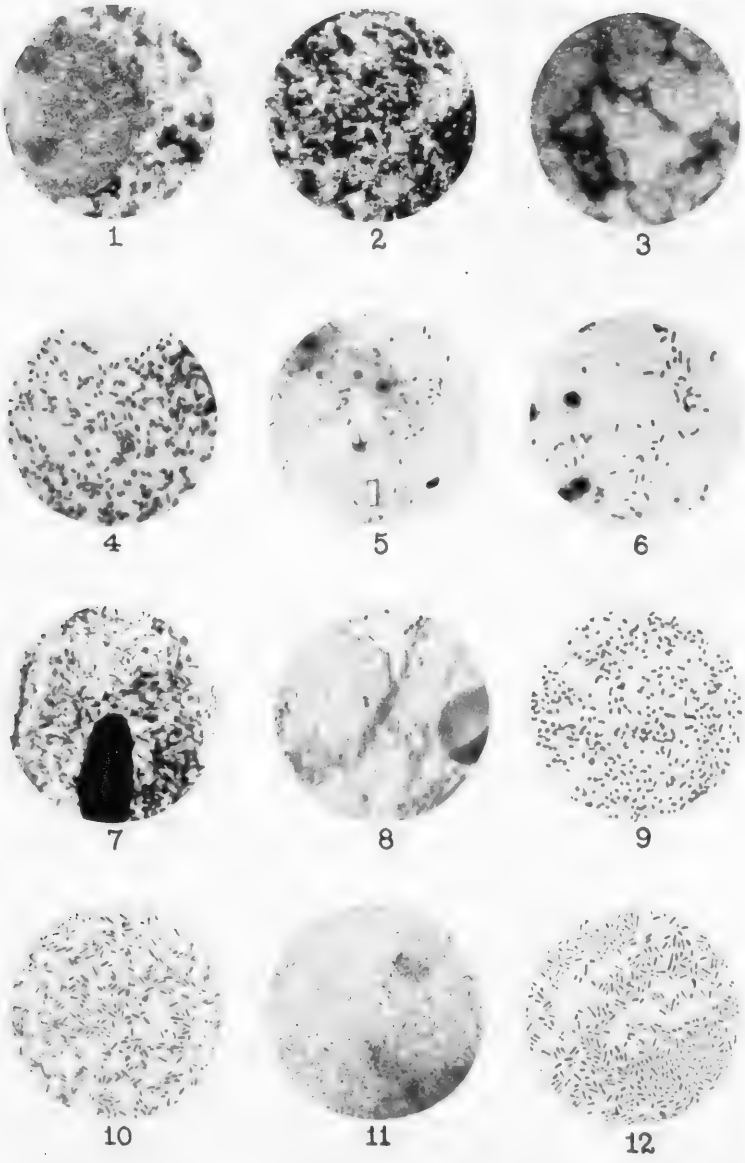
FIG. 34. Same organism grown on ascites broth at 37°C. for 3 days.

FIG. 35. Same organism grown on tissue broth at 37°C. for 8 days.

These photographs show the close resemblance of the forms found in cultures of *B. rickettsiformis* grown under different conditions to the spotted fever organism in mammalian tissues, although *B. rickettsiformis* is not pathogenically or immunologically related to the causative organism of spotted fever.

PLATE 18.

Magnification × 1000. Giemsa's stain.
FIG. 36. *B. pseudoxerosis* grown on agar slant for 3 days at 26°C.
FIGS. 37 and 38. Same organism grown in broth for 5 days at 26°C.
FIGS. 39 and 40. *B. equidistans* grown on agar slant for 3 days at 26°C.
FIG. 41. Same organism grown on broth for 5 days at 26°C.



(Noguchi: *Rickettsia*-like microorganisms.)

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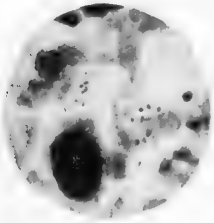

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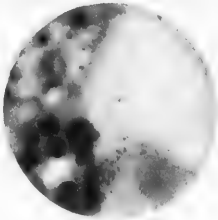

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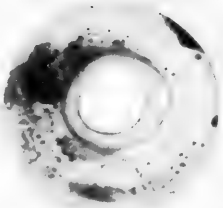

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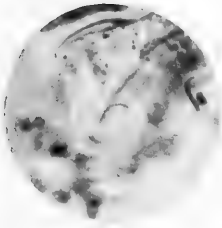

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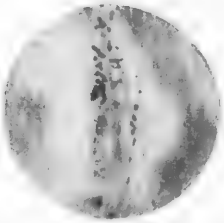

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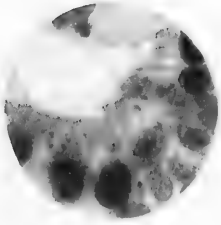

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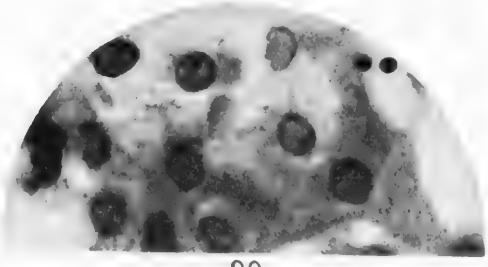

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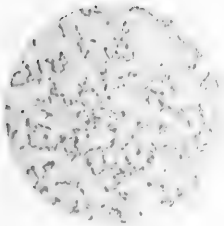

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(Noguchi: *Rickettsia*-like microorganisms.)

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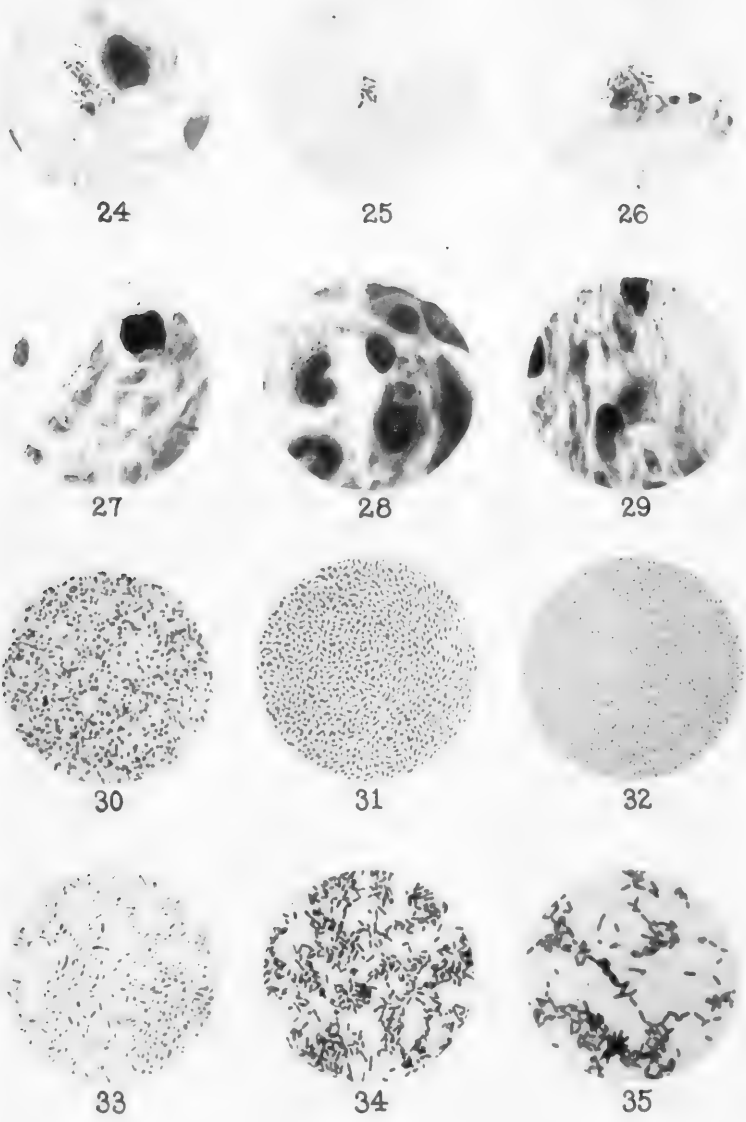

(Noguchi: *Rickettsia*-like microorganisms.)

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(Noguchi: *Rickettsia* like microorganisms.)

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EXPERIMENTAL BONE MARROW REACTIONS.

I. Anemia Produced by Collargol.

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Plate 19.

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As a part of an investigation of the processes which underlie the various types of anemia, the attempt is made in the present study to analyze the factors of blood formation and blood destruction in a form of experimental anemia produced by collargol or colloidal silver.

Colloidal silver was employed experimentally by Cohn (1) in 1904. He demonstrated that the silver particles were engulfed in phagocytic cells in the same manner as other substances existing in a finely subdivided state. Since that time numerous experiments have established the fact that many varieties of particulate matter, when introduced in the blood stream, are deposited in phagocytic cells supposedly of reticular and endothelial origin. Because of the functional similarity of these cells, Aschoff (2*a*) introduced the all including term "reticuloendothelial system," but Sabin (3*a*) has recently differentiated the reticulum and endothelium and their respective genetic phagocytic derivatives into "monocytes" and "clasmatocytes." Throughout this paper the terminology of Sabin is adhered to.

Kiyono (4) studied the difference of the deposition of collargol and carmine. He found that collargol passes very slowly through the vessel wall, carmine more quickly. Hence it was much more difficult to make the clasmatocytes of the interstitium of the internal organs take up silver granules even after repeated injections. He concluded, therefore, that collargol has a more selective action than carmine on the endothelium of the so called blood-forming organs. Similar results were obtained by Tschaschin (5). The avidity of Kupffer's cells for colloidal silver in comparison with other colloidal metals has been noted by Brötz (6). A difference of deposition and distribution of colloidal substances has also been observed by Evans (7*a*), Evans and Schulemann (7*b*), Nissen (8), and Drinker, Shaw, and Drinker (9).

Interest in the phagocytosis of particulate matter has been superseded by investigation of other functions of these widely distributed phagocytic cells. Ribbert (10), the first one to demonstrate these cells by use of vital dyes, as early as

1904, suggested that they might be of importance in metabolic activity. More recent efforts have been directed towards two main problems; namely, the site of bile pigment and antibody formation.

Very little attention has been devoted to the effects of particulate matter on the bone marrow from the point of view of blood formation and blood destruction. For this purpose, colloidal silver was employed in the present study. This colloid is not easily eliminated from the body, lodging in the so called hematopoietic organs; *i.e.*, mainly in liver, spleen, bone marrow, and lymph nodes. It is evident that colloids easily dispersed and eliminated from the body may differ in the results produced.

Material and Methods.

The conclusions of this paper are based on observations of 25 rabbits and 46 rats, 20 of the rats being splenectomized. The animals all appeared to be in excellent health.

The collargol or colloidal silver employed is said to contain 78 per cent of metallic silver and a small percentage of egg albumin and its oxidation product (11). It is manufactured by the Heyden Chemical Works, and distributed by Schering and Glatz, New York. The size of the particles has been determined by Bechhold (12) to average 20 millimicra ($\mu\mu$), the individual particle consisting of aggregates of metallic silver and the protective colloid. The concentration of the colloidal suspension, which was made up in small doses in sterile distilled water and filtered immediately before use, varied between 0.1 and 2 per cent. Physiological saline as a solvent was tried, but a white precipitate was formed on standing and the suspension changed color. The same observation was made by Voigt (13*a*).

As a rule injections were given intravenously every 2nd day. Ear veins were used in the rabbits and tail veins and femoral veins in the rats. A small amount of ether was given the rats and their skin was nicked before inserting the needle.

Frequent examinations were made of the peripheral blood. For blood counts the median artery of the ear of the rabbit was pricked with a needle and the tail of the rat snipped with a pair of sharp scissors. The hemoglobin was read in a Duboscq colorimeter against the Newcomer standard. Vital stains with brilliant cresyl blue were made in all instances and the smears counterstained with Wright's stain.

Some of the rabbits were killed with a blow on the head and subsequent bleeding, after one or more injections; others succumbed to the anemia produced. The surviving rats were killed by ether and bleeding. All tissues were fixed in Zenker's fluid, stained with watery eosin 5 per cent, and counterstained with

methylene blue. Ammonium sulfide and potassium ferricyanide were used for iron stain. The latter dissolves out the silver from the cells, thus removing a substance which otherwise would have obscured any iron present in the tissue.

The bone marrow specimens of the rabbits were consistently taken from the right femur. The cross-sections studied were obtained from the upper third at the level of one of the nutrient arteries entering the bone about ½ inch below the head of the femur. Longitudinal sections were taken from the middle and the lower end of the femur. In rats the entire bone marrow from the right femur was fixed.

Normal animals and animals receiving distilled water intravenously were studied as controls.

Experimental Observations on Rabbits.

The most satisfactory results were obtained with rabbits as their femoral bone marrow is normally more fatty and less hyperplastic than that of rats. Of the twenty-five rabbits studied, eleven received colloidal silver in sufficient amounts to produce a fatal anemia. Of these, eight died and three were killed when moribund. The other fourteen animals were killed at various intervals before a fatal anemia had developed.

Three fairly well defined stages followed the injection of colloidal silver into rabbits; namely, an initial stage of erythropoietic hyperplasia, an intermediary stage with myeloid hyperplasia predominating, and a final, comparatively aplastic, stage.

1. The stage of erythropoietic hyperplasia may be illustrated by two groups of experiments.

(*a*) This included three animals killed after one dose of collargol.

Rabbit H-45, a female weighing 1843 gm., received 4 cc. of a 2 per cent suspension, and was killed 24 hours after the injection. The autopsy and microscopical examination gave essentially normal findings except for the deposition of collargol to a small extent in endothelial cells and clasmatocytes.

Rabbit H–3, a female weighing 1498 gm., also received one dose of 6 cc. of a 0.4 per cent collargol suspension, but was killed 7 days after the administration. Microscopical examination of the marrow revealed a slight but definite endothelial and erythropoietic stimulation. Not only the endothelium lining the patent sinuses showed this early stimulation, but also the endothelial cells between the fat cells were large and swollen, many of them indicating a potential communication between two widely open sinuses. The picture seen in this early stimulated marrow resembles the description given by Doan, Cunningham, and Sabin

(14*a*) of early stimulated marrow in pigeons and rabbits. It also illustrates beautifully the intersinusoidal capillaries described by Doan (14*b*) and their opening up between the fat cells when the marrow is stimulated. A fair number of clasmatocytes and endothelial cells contained small amounts of collargol, many of them situated in clusters of erythroblasts and normoblasts. A few myelocytes showed mitosis, but erythroblastic stimulation predominated. In Fig. 1 is shown an isolated focus illustrating endothelial hypertrophy and mitosis in close approximation to megaloblasts and clasmatocytes containing small amounts of colloidal silver.

(*b*) Animals receiving more than one injection showed a greater degree of hyperplasia. After a certain number of injections, however, varying in different animals, a hypoplasia was produced.

Rabbit H-13, a large male weighing 2755 gm., was killed when the peripheral blood showed marked erythropoietic activity (22 normoblasts were seen in stained specimen while 100 white blood cells were counted), without appreciable decrease of red cells. The total amount of collargol administered was 174 mg. per kilo. The bone marrow showed a more advanced stage of erythropoietic stimulation than that of Rabbit H-3. This hyperplasia included the endothelial cells, which were definitely swollen in most places, with an increase of the number of foci of megaloblasts, erythroblasts, and normoblasts. The myeloid elements were increased only to a moderate extent. Architecturally, the bone marrow was intact, but many fat cells appeared shrunken with consequent increase of the interstices between the fat cells. The venous sinuses were dilated but there was no apparent extravasation of the blood into the parenchyma. The collargol was deposited mainly in clasmatocytes, and, in very small amounts, in the endothelial cells.

The above experiments show the first stage of the reaction following the intravenous injections of colloidal silver. A considerable amount of the silver was taken up by the phagocytic cells of the liver, spleen, and lymph nodes, and only a small amount reached the bone marrow where it produced a slight stimulation of the endothelial cells with an increase of clasmatocytes and cells of the erythrocytic series. Evidence of this erythropoietic stimulation was found in the outpouring of normoblasts in the peripheral blood. It is interesting to note that an outpouring of young cells occurred at some stage in all the animals receiving moderately large amounts of collargol intravenously, even before any decrease of blood cells had taken place. Stimulation of the myeloid series was less marked.

A similar stimulation of the bone marrow has been observed incidentally after injections of other colloids.

Nissen (8) found a hyperplastic bone marrow after five doses of "elektroferrol" and five doses of carmine, with erythroblastic hyperplasia predominating. Doan, Cunningham, and Sabin (14*a*) describe endothelial and reticular hypertrophy in pigeons after injections of three doses of trypan blue and carmine. A pigeon receiving trypan blue when fasted did not develop a hypoplastic bone marrow as the result of inanition, but the dye had a stimulating effect on cell production in the marrow, and a hyperplastic bone marrow was obtained. The stimulating effect of the dye seemed to be dominant over the influence of inanition, which usually decreases hematopoiesis in pigeons.

2. The second or intermediary stage is represented by Rabbits H-35, H-34, and H-47.

Rabbit H-35, female weighing 1850 gm., received the same amount of collargol per kilo body weight as Rabbit H-13, given, however, in four large doses. The peripheral blood, just before the animal was sacrificed, showed practically no erythropoietic stimulation and no anemia. Microscopically, the marrow showed a hyperplasia, the cellularity being approximately the same as in Rabbit H-13. The interesting feature in this marrow was the definite shifting of the stimulation to the myeloid series. The clasmatocytes were prominent, loaded with collargol, but collargol was also deposited in the endothelium lining the wide open sinuses. The endothelium showed very little activity, and there was practically no evidence of erythroblastic stimulation. The fat cells were shrunken in size and irregular in outline.

Rabbit H-34, of about the same weight and receiving the same treatment as No. H-35, showed marked erythropoietic stimulation, indicated in the peripheral blood by the number of normoblasts present (20 normoblasts seen in stained specimen while 100 white blood cells were counted). Red blood cells were normal (5,100,000). The bone marrow was intensely hyperplastic; this hyperplasia included both the erythrocyte and the myeloid series, the increase of the myeloid elements predominating.

Rabbit H-47, a female, was given 140 mg. per kilo body weight, administered in seven doses. When the animal was killed there were no normoblasts in the peripheral blood and the red blood cells were decreased (3,730,000). The bone marrow picture is practically identical with that of No. H-35 described above.

These animals represent a more advanced phase of the effects of colloidal silver on the bone marrow. In the case of No. H-35, owing to larger dosage and perhaps somewhat to individual susceptibility, the phase of erythrocyte hyperplasia was passed and there was little

or no evidence of hyperplasia of the endothelium. Rabbit H-34 possibly represents a transition between the first and second stage, while Rabbit H-47, as shown by the red blood cell count, was rapidly approaching the final stage.

In all the animals so far described as showing red cell stimulation (Group 1, and No. H-34, Group 2), there was a coincident hyperplasia of endothelial cells, many of which were normal in appearance and without phagocyted particles. In the remaining animals of Group 2, however, many of the endothelial cells contained collargol. Fat absorption and myeloblastic hyperplasia have been noticed by Nissen (8) after five doses of "elektrokollargol." Doan, Cunningham, and Sabin (14*a*) found a mild myeloid hyperplasia with very little red blood cell activity in a rabbit subjected to fifteen intravenous injections of trypan blue. The endothelium in this case contained trypan blue in contrast to that of animals receiving only a few injections of the dye.

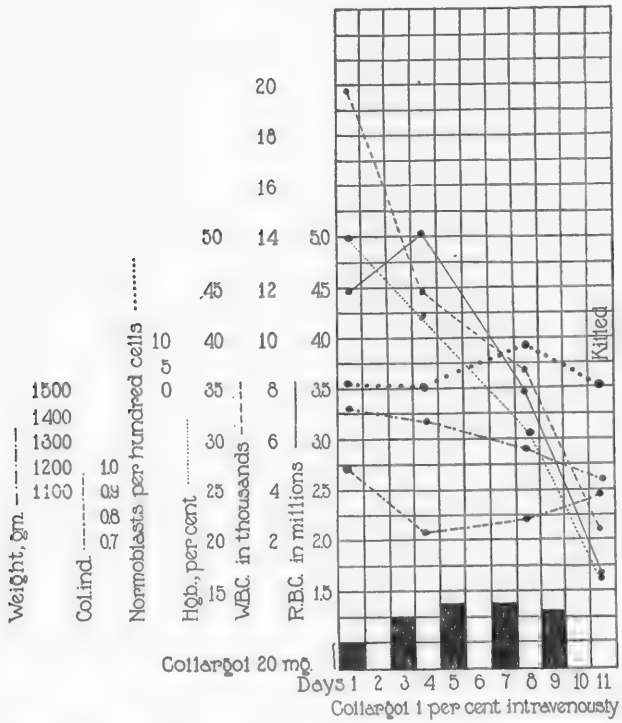
3. The final stage is shown by the animals which were killed when moribund as the result of the collargol injections or which died spontaneously with signs of grave aplastic anemia. Their course is best represented by charts.

Rabbit H-16, Chart 1, a young female, showed an unusually rapid course. On the 2nd day after the last dose the animal was moribund. On microscopical examination of the bone marrow all normal structure was gone, and the fat cells were replaced by an acidophilic fibrin-like ground substance or stroma with few developing cells. The marrow cells were definitely decreased and the few cells remaining were in lines in close relation to the blood vessels. The collargol was deposited in both clasmatocytes and endothelial cells. The endothelial cells lining the patent sinuses and those in between the sinuses were hypertrophied. An occasional megaloblast was seen; most of the red blood cells present, however, were in the erythroblastic stage, while the normoblastic elements were practically absent. The more mature myelocytes had largely disappeared, but a few foci of young myelocytes were seen here and there. The spleen was loaded with iron, which may be due to an early stimulation of the endothelial cells and their derivatives to red blood cell phagocytosis, as has been observed by Motohashi (15) after injection of collargol.

The rapidly fatal course of No. H-16, with the marked diminution of the cellular elements in the peripheral blood, suggests that, in addition to the depression of blood formation, a very active destruction must have taken place, perhaps by means of red blood cell

phagocytosis in the spleen. This, however, was not a prominent feature in the other animals with a fatal course.

Rabbit H-27, Chart 2, a male, had a more protracted course and lived 6 days after the last injection of collargol. The day before exitus the hemoglobin was



In Charts 1 to 4 the number of normoblasts given is the number per 100 *white blood cells*.

CHART 1. Rabbit H-16. Unusually rapid course in young animal. The spleen was filled with iron, which suggests increased blood destruction in addition to depression of blood formation. Striking evidences of blood destruction not found in other animals similarly treated.

12 per cent, red blood cells 1,200,000, and leucocytes 4100. The bone marrow was grossly grayish and firm. Microscopically, the fat cells showed signs of shrinkage, the widened interstices being filled with the same eosin-staining ground substance as in No. H-16. The collargol was aggregated in large masses, consisting, apparently, of several clasmatocytes filled with pigment. Around these masses

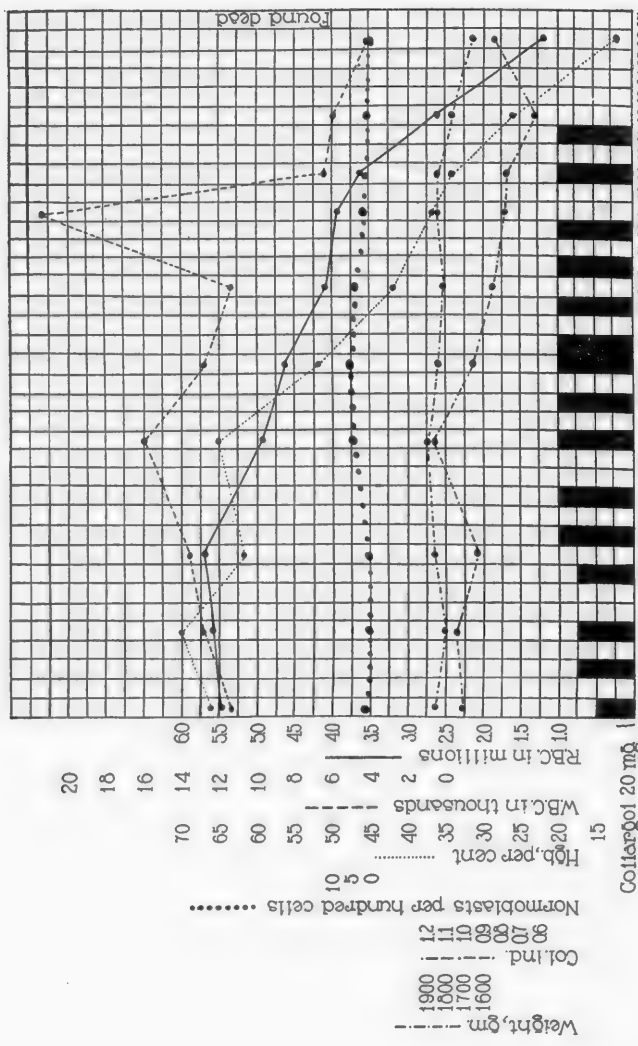

Found dead
RBC. in millions
WBC. in thousands
Hgb. per cent
Normoblasts per hundred cells
Col. Ind.
Weight, gm.
Days 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
Collargol 20 mg.
Collargol 2 per cent intravenously

CHART 2. Course of Rabbit H-27, an older animal, which died at a time when the hemoglobin was 12 per cent, red blood cells 1,200,000. Striking hypoplasia of the bone marrow with hypertrophy of the endothelium of the vessels of the mesenteric lymph nodes.

there was no evidence of inflammatory reaction or encapsulation. A moderate amount of collargol was found in the endothelium which, in places, showed hypertrophy. A small number of erythroblasts were present with a moderate number of normoblasts. A moderate number of myelocytes of the later stage were seen. The cells of both the erythrocytic and myeloid series were definitely decreased. This animal showed a decrease of the number of megalocaryocytes. This was noted in two other animals, in one of which death was precipitated by hemorrhages from the intestinal mucosa.

The bone marrow of No. H-27 gave very little evidence of endothelial hypertrophy or erythropoiesis. This animal, however, showed a proliferation of the endothelium in the small veins of the mesenteric lymph nodes. The endothelium in these places did not show any collargol in contrast to the endothelium of the sinuses, which contained a moderate amount of silver. The phase of division was not caught in this section and there are no evidences in what direction these new cells were differentiating.

Rabbit H-29, male, Chart 3, was killed when moribund 6 days after the last dose. The peripheral blood just before death showed: hemoglobin 18 per cent, red blood cells 2,100,000, white blood cells 3500. Microscopical examination of the bone marrow showed essentially the same as in No. H-27. This animal illustrates beautifully the outpouring of normoblasts (see chart) beginning before any decrease of red blood cells has taken place and stopping when the peripheral blood shows a decrease of red blood cells—a time when one would expect to find normoblasts and other evidences of active blood formation.

Rabbit H-1 is noteworthy from two points of view; namely, the more chronic course with more severe bone marrow aplasia, and the failure to regenerate although life was prolonged for 18 days after the last injection. In all 660 mg. per kilo body weight were administered over a period of 41 days. 8 days before death, the hemoglobin was 22 per cent, red blood cells 2,480,000, leucocytes 10,150 with 1 normoblast in 100 leucocytes counted. The marrow showed the same eosin-staining fibrillar ground substance found in this group of animals and marked cellular aplasia except at the periphery of the cross-section, where there was a cellular area not containing collargol. An enormous number of clasmatocytes contained collargol, in comparison with which the endothelial cells contained but little colloidal silver. The endothelium showed no signs of hypertrophy and most of the endothelial cells present were lining the dilated venous sinuses and capillaries which persisted throughout. The architecture and the striking diminution in number of cells in this bone marrow are well shown in Fig. 2.

In all the animals the number of blood cells remained normal for a considerable length of time after the injection of collargol had begun.

To see whether the animals would recover if the injections were stopped as soon as the red blood cells began to decrease and before a definite anemia had developed, the following experiment was performed. Five rabbits of about the same weight were given eight injections of 4 cc. of 2 per cent collargol each. One animal was killed 6 hours after the last dose, two animals were killed 4 days after the last dose, and two animals succumbed spontaneously.

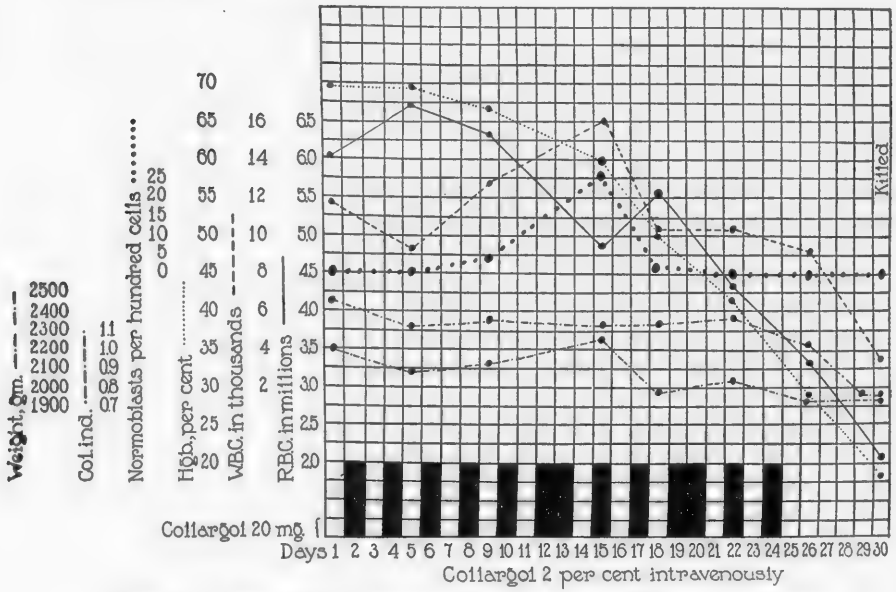

Weight, gm.
Col. ind.
Normoblasts per hundred cells
Hgb. per cent
W.B.C. in thousands
R.B.C. in millions
Killed
Collargol 20 mg.
Days 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Collargol 2 per cent intravenously

CHART 3. Rabbit H-29, illustrating the outpouring of normoblasts beginning before any decrease of red blood cells has taken place and stopping when the peripheral blood showed a decrease of red blood cells.

The course of three of these experiments is represented graphically in Chart 4, *a*, *b*, and *c*.

Rabbit H-52, female, Chart 4, *a*, was killed 6 hours after the last dose. As may be seen from the chart, the red blood cells showed some decrease in number. The bone marrow showed a moderate hyperplasia of both the erythrocyte and myeloid series, but the latter predominated. One may classify this marrow as belonging to the second or intermediary group. The general architecture and cellularity are represented in Fig. 3.

In Rabbit H-33, female, Chart 4, *b*, killed 4 days after the last dose of collargol, the marrow showed a comparatively normal structure as far as fat cells,

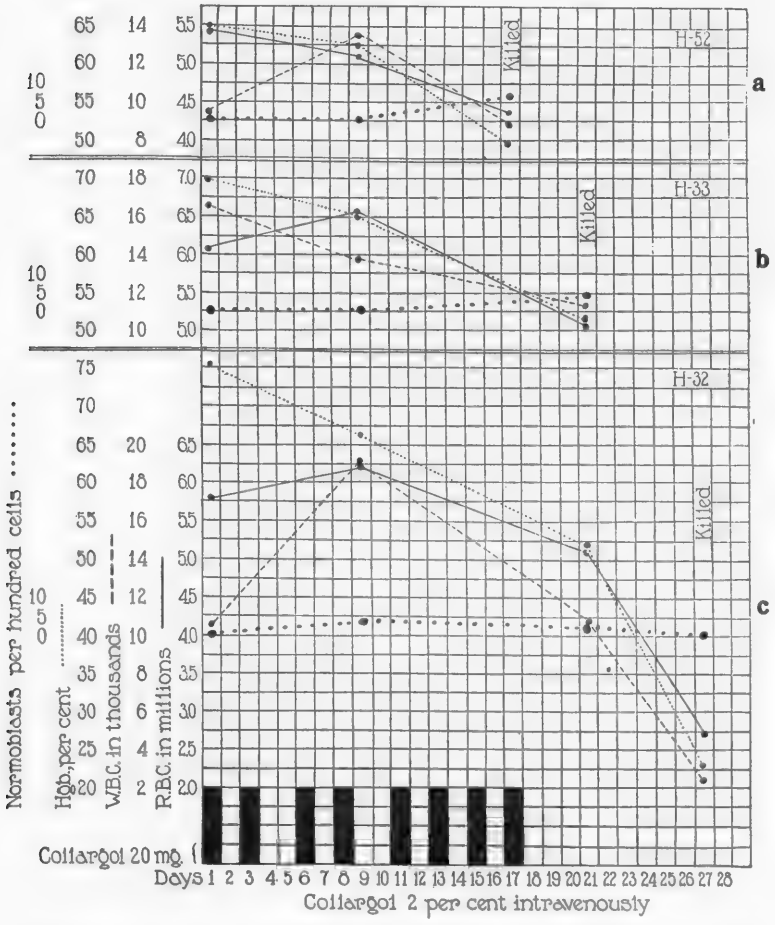


CHART 4, *a* to *c*. (*a*) Rabbit H-52, killed 6 hours after the last dose. For architecture and cellularity of bone marrow see Fig. 3. (*b*) Rabbit H-33 killed 4 days after the last dose at a time when blood was beginning to show some decrease in erythrocytes and hemoglobin. The marked decrease in marrow cells and deposition of collargol in the endothelium is illustrated in Fig. 4. (*c*) Rabbit H-32 killed when moribund. Bone marrow similar to that in Fig. 4.

blood vessels, and megalocaryocytes were concerned. There was, however, a marked decrease in cells of both the myeloid and erythroid series. The collargol was deposited in considerable amounts in the endothelium, which did not seem hypertrophied and was present as well in large amounts in clasmatocytes.

The bone marrow of Rabbit H-32, female, Chart 4, *c*, killed when moribund, showed very few marrow cells and fat cells decreased in number and shrunken in size. The blood capillaries and sinuses were well maintained, practically all of them lined with a hypertrophied endothelium containing collargol. An occasional focus of red blood cells was present, mainly in the erythroblastic stage with a rare megaloblast. A few groups of normoblasts also remained. The myelocytes were scant, most of them being immature forms.

Fig. 4 illustrates the deposition of collargol in the endothelium as well as the general structure and cellularity of the marrow of No. H-33. In several of the animals receiving 2 per cent collargol, the fat cells did not disappear to the same extent as in those animals receiving less concentrated solutions. This seems to indicate that the stage of stimulation was less protracted in those animals receiving the higher concentration of the silver suspension. Ribadeau-Dumas and Debré (16) have also noticed that the bone marrow reaction in rabbits was modified towards hyperplasia by weak and moderate doses of collargol, but that the reaction was less intense after concentrated doses.

The marrow of the other two of the five animals similarly treated was practically the same as that of those described above.

The animals just described (Nos. H-52, H-33, and H-32) present several interesting points. The administration of collargol was stopped after eight injections and before the animals had begun to show any appreciable anemia. The bone marrow of the rabbit (No. H-52) killed 6 hours after the last dose showed considerable signs of activity and stimulation, and was in marked contrast to that of the animals allowed to live without further administration of collargol. This suggests that the action of the colloidal silver continues after the administration has been stopped, probably by the mechanism of disintegration of the clasmatocytes containing collargol and the engulfing of the particles thus set free by new clasmatocytes and endothelial cells. The bone marrow of the animals killed 4 days after the last dose showed a greater depression of the erythropoietic function than that of the animals allowed to live until moribund. The striking coincidence of a bone marrow aplasia without any anemia in the peripheral blood indicates that collargol injures the bone marrow without affecting the formed red cells. After the injury is accomplished, the animal continues to live until the circulating formed blood elements

decrease in the course of normal blood destruction to a point incompatible with life. There is, however, some slight effort on the part of the bone marrow to produce blood cells, evidenced by the occasional foci of erythroblasts, although this effort is wholly inadequate and the animal dies with signs of grave anemia.

The peripheral blood of all the animals in which a fatal anemia was produced showed a marked anisocytosis towards the end, with pronounced central pallor and a tendency to microcytosis. Platelets, as noted in the smears, were abundant. Normally, the blood of rabbits shows some polychromatophilia and reticulated cells. Discounting this, all the animals receiving several doses of collargol showed certain constant characteristics; namely, the progressive increase of young cells in the peripheral circulation followed by decrease, until finally neither polychromatophilia, reticulated cells, nor normoblasts were present. This later stage was seen only in animals running a fatal course. The color index in most instances was decreased.

The autopsy findings, other than those already discussed, were uniform in the eleven animals which died from anemia. As was to be expected, large accumulations of collargol were found in phagocytic cells in the liver, spleen, and lymph nodes. In the kidneys the collargol was deposited in the endothelium of the capillaries, but very little was found in the glomeruli or in the epithelium of the tubules.

Voigt (13*b*), studying the fate of collargol in rabbits, found that kidneys and intestines contained silver. He examined urine and feces but could not demonstrate the presence of silver.

That the distilled water used as solvent for the collargol played no part in the results produced was conclusively proven by findings in control animals.

Experimental Observations on Rats.

A. Normal Rats.

As indicated above, rats did not prove to be as satisfactory experimental animals as rabbits. Many factors probably contributed to this, the most important being the very active hematopoietic function in rats, and the comparatively large spleen and liver which seem to

form an efficient protective barrier to substances injected in the blood stream. Nevertheless, in spite of this, anemia, secondary in type, was produced in all animals receiving a moderate amount of collargol, and a few animals died as a result of this anemia.

While the red blood cells and hemoglobin of the rabbits gradually decreased if the injections were stopped when the initial decrease had begun, the rats recovered promptly as soon as the injections were discontinued, unless hemoglobin and red blood cells had reached a dangerously low point. Therefore, in order to produce an anemia, injections had to be continued. In the later stages the administration became increasingly precarious, and the experiments were interrupted in many cases by the animals dying under the ether or shortly after.

Chart 5 illustrates the course of Rat R-26, which died 6 days after the last injection with signs of grave anemia. The bone marrow in this, as well as in many other animals, could not be analyzed as no definite structure could be made out, but there was a relative decrease of cellular elements and most of the cells present were young forms. The megalocaryocytes were definitely decreased.

Six rats were injected with a 0.1 per cent suspension, the lowest concentration employed in these experiments. The course of all these animals seems to point to a direct relationship between the amount of collargol injected and the anemia produced.

That the general experimental procedure and the distilled water used for solvent of the collargol played little or no part in the production of the anemia was shown by the five controls.

In comparing rats and rabbits certain differences stand out. The bone marrow of the rabbit contained more collargol after the administration of comparatively smaller amounts, and the degree of depression of the hematopoietic function was more marked in rabbits than in rats. Leucopenia, a comparatively constant finding toward the end in rabbits, was not obtained in rats. Iron was always found in the spleen of rabbits dying from anemia, but in rats it was practically absent after the administration of a small number of medium sized injections in spite of the fact that the spleen of the adult rat is normally loaded with iron.

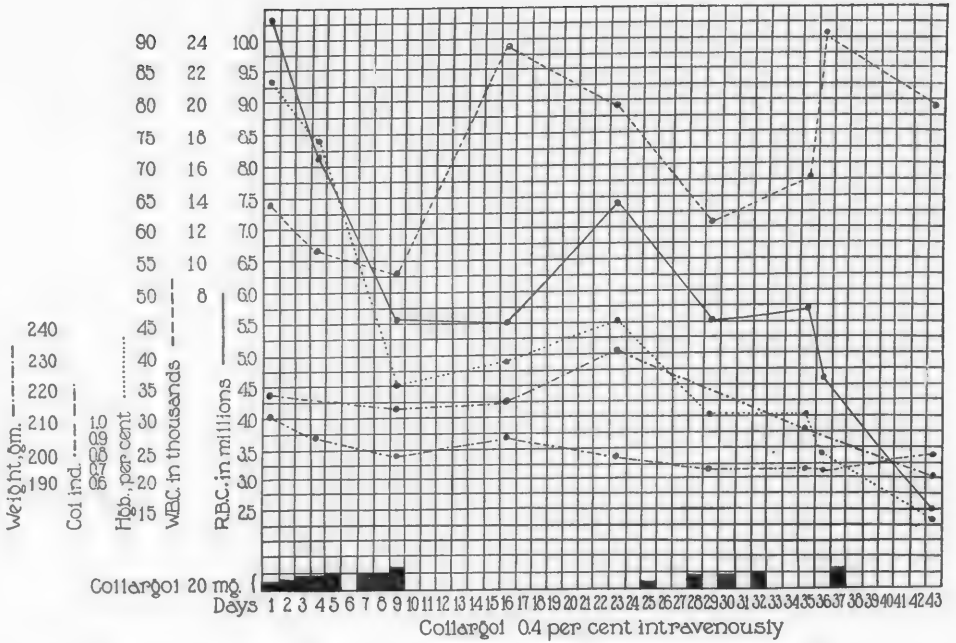

Weight, gm.
Col ind.
Hgb., per cent
WBC. in thousands
RBC. in millions
Collargol 20 mg.
Days 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43
Collargol 0.4 per cent intravenously

CHART 5. Course of Rat R-26. Death occurred 6 days after the last injection. Signs of grave anemia.

B. Splenectomized Rats.

To see whether it would be possible to cause collargol to be deposited in larger amounts in the bone marrow, a series of rats was splenectomized.

The general course of the splenectomized rats did not differ from that of the non-splenectomized ones. As in the non-splenectomized series, animals were killed at different stages. As far as could be determined, there was no appreciable difference in the degree of anemia produced in the same time interval with the same amount of collargol, nor did there seem to be any marked increase in the amount of collargol deposited in the bone marrow.

The findings described above seem to indicate that the chief site of clasmatocyte formation differs in animals of different species. In the rabbit, the bone marrow participates to a considerable extent, while in rats, after the removal of the large spleen, most of the compensatory function is shifted to the liver. This probably holds true for non-splenectomized animals also, after overloading the spleen with particulate matter.

That the site of compensatory activity varies in different animals has been shown by several observers.

Pearce and Austin (17) found that in splenectomized dogs a compensatory activity took place in lymph nodes and liver. In splenectomized rats, Lepehne (18) found compensatory activity in Kupffer's cells in the liver. Motohashi (15), in a series of splenectomized rabbits, observed that hemophagic activity of the spleen in rabbits had been transferred to the bone marrow and, to a lesser extent, to the liver. This compensatory function has been emphasized by Krumbhaar (19).

DISCUSSION.

The above experiments indicate that the administration of comparatively small amounts of collargol produced, first, a stimulation of the endothelium, of the erythrocyte series, and, to some extent, of the myeloid series; and, second, the accumulation of the collargol mainly in the clasmatocytes and, to a much less extent, in the endothelial cells. The animal's health remained unimpaired and the blood counts were normal.

If larger amounts of collargol were administered over a longer period of time, the bone marrow changed from a hyperplastic to a compara-

tively aplastic one, and, in addition to the clasmatocytes, the endothelial cells took up a considerable amount of collargol. At this stage, there was a very marked anemia and scarcely any evidence of red cell formation in the bone marrow. The myeloid series showed more activity and the leucocytes in the peripheral blood decreased in number only towards the end.

That collargol produces similar results in man has been shown by accidental fatalities from therapeutic use.

Two cases were reported by Herzog and Roscher (20*a*), in 1922, in which, after large doses of collargol, constitutional symptoms appeared, both cases ending fatally, one in 9 days and the other in 16 days. Leucocytosis and outpouring of normoblasts occurred in these cases just as in the rabbits, before anemia had developed. In the chronic case leucocytosis was replaced by leucopenia before the end. The prominent feature, however, was the purpura and the bleeding from the mucous membranes.

Bleeding was not seen in my series of animals except in Rabbit H-29, which showed extensive hemorrhages from the intestinal mucosa, with a marked diminution of the megalocaryocytes in the bone marrow. The authors considered the human cases similar to those of benzene and radium poisoning, interpreting their findings as an initial stimulation of the bone marrow followed by depression.

As the above cases had received, in addition to collargol, two doses of salvarsan, Herzog and Roscher (20*b*), in an attempt to find out the cause of death, injected one rabbit with collargol, administering 912 mg. in twelve doses, distributed over a period of 27 days. The animal died with signs of severe anemia and their results are similar to those of the experiments described above, with this difference, that no leucopenia was obtained and myeloid metaplasia was observed in the spleen.

The explanation of the anemia produced by the injections of collargol must, in the light of the experiments described in this article, be sought in the bone marrow. The coincidence of a stimulated endothelium, with erythropoietic activity, and of a quiescent endothelium, with decrease or lack of erythropoiesis, is, to say the least, striking, while myeloid activity seems to be independent of the endothelial changes. The suggestion, therefore, presents itself that a close relation must exist between the erythrocytes and the endothelium and its derivatives.

As the result of recent research, the theory that the endothelium is the progenitor of the clasmatocytes and the red blood cells is gaining ground. The

most recent investigation and the most convincing proof of the endothelial origin of the clasmatocytes, first suggested by Mallory (21) in 1898, are offered by Sabin (3*b*).

Due to the researches of Sabin *et al.*, new evidence has been brought forth also in support of the theory of the endothelial origin of the erythrocytes. Sabin (3*c*), in 1920, demonstrated this genetic relationship in the chick embryo, while Doan (14*c*) in 1922, analyzing the bone marrow of the adult pigeon, and Cunningham and Doan (22*a*), studying the mammalian bone marrow, drew the same conclusions. From this analysis the conclusion was also drawn that white blood cells arise from the reticular cells extravascularly in the intervascular parenchyma (Cunningham, Sabin, and Doan (22*b*)).

Accepting the evidences offered of the endothelial origin of the clasmatocytes and the red blood cells as a working hypothesis, it ought to be theoretically possible to influence functionally this widespread system of endothelial cells and indirectly the production of both red blood cells and clasmatocytes.

As seen in the experiments described in this paper, the first action of collargol is a stimulation of the bone marrow with outpouring of young erythrocytes and normoblasts. This stimulation is observed after the injection of many colloidal substances. The increased activity is, however, not confined to the erythrocytic series of cells, but also includes the clasmatocytes.

Experimentally, large phagocytic cells were produced by Evans and Winternitz (7*c*), in 1911, Aschoff and Kiyono, in 1913 (2*b*), and Kiyono, in 1914 (4), who found these cells in the circulation after repeated injections of carmine, isamine blue, trypan blue, and collargol. Simpson (23) confirmed these observations, employing various colloidal suspensions.

It is of significance to note that the stimulation caused by the colloidal substances includes both red blood cells and clasmatocytes. Theoretically, one may conceive that if the rate of excretion of the colloid injected is in any way commensurable with the rate of introduction, a continued stimulation of the endothelium could be obtained with new formation of erythrocytes and clasmatocytes. If, however, colloidal substances with a low rate of diffusibility, such as collargol, are injected continuously, the development of the clasmatocytes from the endothelium becomes paramount at the expense of the red blood cell formation and finally the endothelium is filled with

particulate matter and becomes genetically inactive; the animal lives only until the already formed blood elements have been destroyed in the daily wear and tear of the red blood cells. The reticulum, which is less phagocytic than the endothelium, is moderately stimulated throughout and not until the end is a leucopenia observed in the peripheral blood.

CONCLUSIONS.

1. The effect produced by the intravenous administration of collargol on the bone marrow of rabbits varies directly with the amount of collargol injected, and three fairly well defined stages could be recognized.

(*a*) An initial stage after comparatively few and small doses, with erythrocytic and endothelial hyperplasia in the bone marrow and with evidences of this stimulation in the peripheral blood in the form of young erythrocytes and normoblasts.

(*b*) An intermediary stage which followed the injection of larger amounts of collargol, and which was characterized by a predominant myeloid hyperplasia.

(*c*) A final stage with marked bone marrow aplasia and with colloidal silver deposited in endothelial cells, as well as in clasmatocytes. This was associated with a high grade anemia with low color index, resembling aplastic anemia in its main features. This stage terminated fatally.

2. There was no evidence of injury to blood cells in the peripheral circulation. The erythrocytic bone marrow aplasia was present before any appreciable decrease of red blood cells was found in the peripheral blood.

3. The results were less clear-cut in a series of rats, but anemia of a similar type was produced in all animals when sufficiently large doses were injected.

4. Splenectomy did not alter the course in rats materially.

5. It is fair to conclude that the cause of the anemia produced may be sought in the deviation of the parental endothelial cell toward clasmatocyte formation at the expense of the development of erythrocytes.

6. It is suggested that the results may be offered in support of the

theory of the endothelial origin of both clasmatocytes and red blood cells.

I am deeply indebted to Dr. Francis W. Peabody for valuable suggestions in the course of the experiments and it gives me great pleasure to acknowledge his and his staff's constant assistance.

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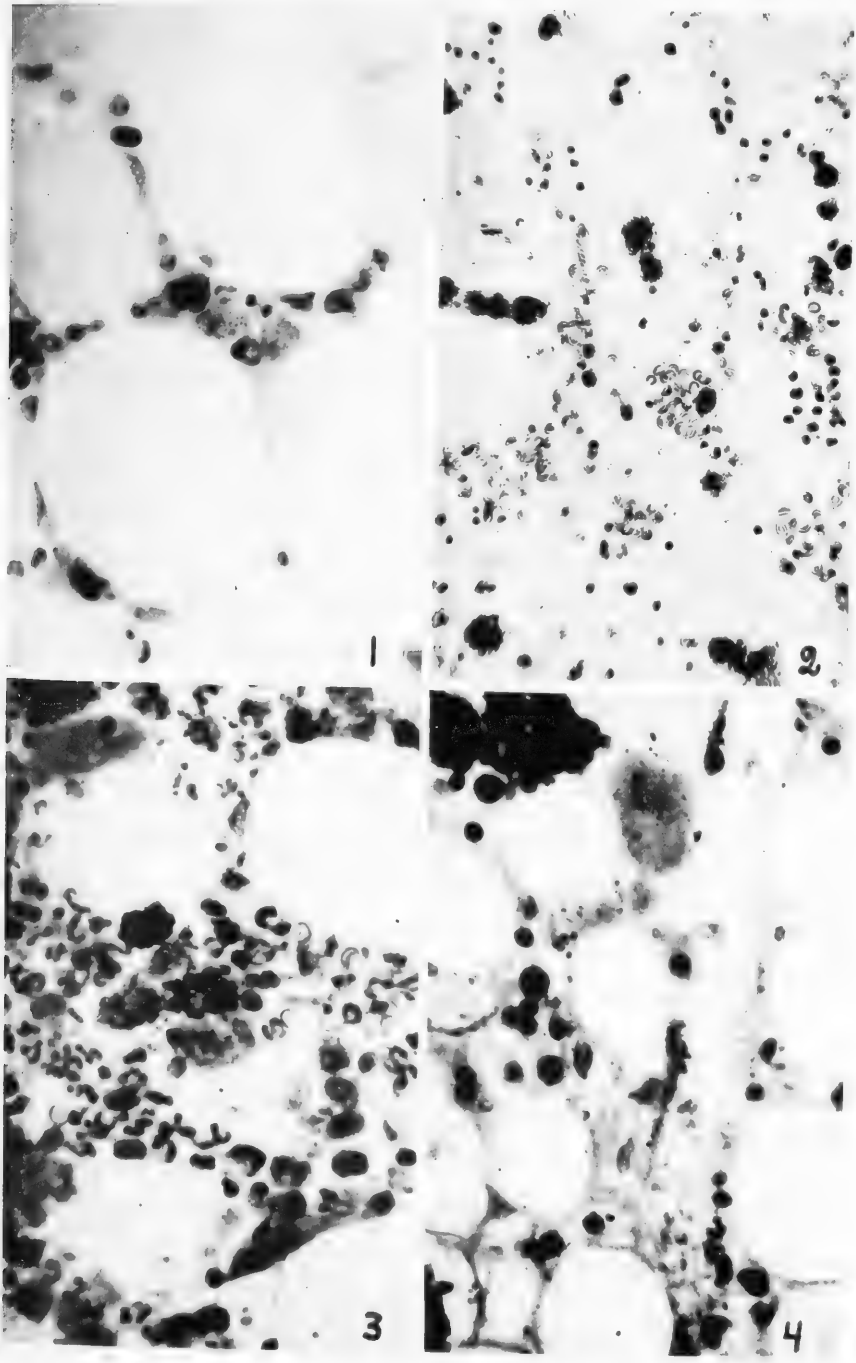
EXPLANATION OF PLATE 19.

FIG. 1. Rabbit H-3. Isolated focus between the fat cells in early stimulated marrow showing hypertrophied endothelium and mitosis adjacent to megaloblast and clasmatocytes containing silver granules. × about 800.

FIG. 2. Rabbit H-1. Bone marrow of animal succumbing 18 days after the last injection of collargol. The normal architecture is gone, being replaced by an eosin-staining ground substance containing many clasmatocytes filled with collargol, but very few cells of the erythromyeloid series. Blood vessels are intact, lined with apparently inactive endothelium. × 370.

FIG. 3. Bone marrow of Rabbit H-52 given eight doses of 2 per cent collargol and killed 6 hours after the last dose. Moderate hyperplasia of both the erythrocyte and myeloid series, the latter predominating. × about 800.

FIG. 4. Bone marrow of Rabbit H-33 subjected to the same treatment as No. H-52, but killed 4 days after the last dose. The peripheral blood showed some decrease in erythrocytes and hemoglobin. Fat cells are intact, but very few cells of either myeloid or erythrocyte series remain. The collargol is deposited in clasmatocytes and in the endothelium lining blood vessels. × about 800.



(Muller: Bone marrow reactions. I.)

UNIV. OF MICH.

EPIDEMIOLOGICAL STUDIES ON RESPIRATORY INFECTIONS OF THE RABBIT.

VII. Pneumonias Associated with Bacterium lepisepticum.

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Plates 20 to 23.

(Received for publication, January 4, 1926.)

An important manifestation of *Bacterium lepisepticum* infection in the rabbit is pneumonia, which occurs spontaneously and is producible experimentally. In the course of our studies on the epidemiology of the disease, we have considered certain other phases of the infection—snuffles, otitis media, and the carrier state. Our present effort has been to classify pathologically the pneumonic lesions and to determine the conditions under which one or another of the type lesions arises. This paper deals with the results of the investigation.

"Spontaneous" Pneumonia.

So called "spontaneous" pleuropneumonia of laboratory rabbits has been observed by many investigators. It is known to be most frequent in the spring and fall, and to occur more often in animals undergoing experiment than in similar ones used as controls.

To emphasize various types of this pneumonia, we have tabulated a group of 124 spontaneous cases, encountered from November, 1924, to May, 1925, at a rabbit farm in New City, New York (Table I). This group is representative of the different pneumonias which have occurred at the Rockefeller Institute laboratories and in other places which we have had under observation.

Although bacteriological determinations were made throughout the period in only half of the animals, so very few cases of pneumonia of other origin were found during this period, and such familiarity

with this infection has been attained, that it may be assumed that all the pneumonias included in this chart were incited by *Bact. lepisepticum.*

No clinical histories were available. The rabbits were sent to the laboratory shortly after death and autopsied. A gross examination was made, cultures were taken when necessary, and suitable tissues were preserved in Helly's fluid for histological examination.

The lungs of twelve rabbits (9.7 per cent) showed little more than congestion. The tissue was dark red in color, bloody on the cut surface, with no gross evidence of consolidation. The pleura was smooth and glistening. Microscopically a general dilatation of the smaller blood vessels and capillaries, especially noticeable between

TABLE I.

Relative Incidence of Various Types of Spontaneous Pneumonias in a Community of Rabbits at New City, N. Y.

Gross pathology.	No. of cases.	Per cent.
Congestion	12	9.7
Red, moist lungs, exudate interstitial, with subpleural and perivascular orientation	17	13.7
Large, grey lungs; rubbery lobar consolidation	29	23.4
Pleuropneumonia	28	22.6
Chronic and acute pneumonia	2	1.6
Multiple small abscesses	27	21.8
Large abscesses	9	7.2
Total	124	100.0

the alveoli and beneath the pleura, was found. All air spaces seemed patent. Occasionally a perivascular collar of eosinophilic and mononuclear cells was noted. Seventeen animals (13.7 per cent) showed large, red lungs, moist and boggy. The pleural surface was usually dulled, the tissue beneath red and firm. On section the cut surface appeared red with granular areas of consolidation spreading diffusely throughout an entire lobe or lung. Microscopically, accumulations of red cells, fibrin, débris, eosinophils, and mononuclears were seen beneath the pleura. The interstitial framework was distorted by dilated capillaries and lymphatics, and by serum and leucocytes. Collars of leucocytes surrounded the blood vessels and filled the perivascular lymphatics. Patchy areas of alveoli were filled with cells and débris. Twenty-nine animals (23.4 per cent) showed massive, grey lungs, hard and firm, with shaggy threads of fibrin covering the pleural surfaces. Purulent fluid in varying amounts was present in each cavity. The alveoli were filled

with necrotic plugs, fibrin, and leucocytes; mononuclear and giant cells were abundant. The lung architecture was obliterated in many places. Frequently, pus cells filled the larger bronchi. Twenty-eight rabbits (22.6 per cent) showed one or both pleural cavities filled with pus, grey and collapsed lungs, and pericarditis. Two cases showed firm, white lungs, with regions of connective tissue proliferation surrounded by hemorrhagic zones, soft and moist. Abscesses were found in 36 cases (29 per cent), sometimes pea-sized, distributed more or less regularly throughout the lungs, or occasionally (7.2 per cent) limited to one or two very large, encapsulated, walnut-sized peduncles, hanging from the pleural surface, forming at the hilum, or adhering to the parietal and visceral pleura. Cheesy pus filled these sacs.

All the strains of *Bact. lepisepticum* recovered from the lungs, nares, or heart's blood of these rabbits were of the mucoid type, of uniform virulence, as tested by intraperitoneal infection in mice. It is intended to present a later report on the behavior of various strains of these bacilli. At present, however, it is sufficient to record that with this uniform type of *Bact. lepisepticum*, pneumonias, arising spontaneously and differing in extent and severity, were associated.

Experimental Pneumonia.

Intranasal Instillation.

Studies of the pneumonias were continued by inoculating rabbits experimentally with *Bact. lepisepticum*. To simulate the natural infection, and to avoid trauma, bacteria were instilled into the nasal passages with a medicine dropper or blunt syringe without needle. Inoculations were made with the Rivers strain, Type D.[1] A 17 hour

[1] The Rivers strain is identical with those cultivated by De Kruif from cases of spontaneous pneumonia (De Kruif, P. H., *J. Exp. Med.*, 1921, xxxiii, 773), and is probably the same type as that usually described in the literature. Although previously not uncommon, this form has been recovered but twice by us from spontaneously infected rabbits during the past 2 years. It appears to be highly pathogenic, but not especially vegetative. The mucoid strains, on the other hand, are at present ubiquitous. Culturally, they are similar to the Rivers and De Kruif strains. Serologically, although mutually related, they differ from the Rivers and De Kruif cultures. Their pathogenicity is less than that of the Rivers type, but their parasitic adaptabilities are greater. Further descriptive details may be found in earlier publications (Webster, L. T., *J. Exp. Med.*, 1925, xli, 571; Webster, L. T., and Baudisch, O., *J. Exp. Med.*, 1925, xlii, 473) in the accompanying paper (Webster, L. T., *J. Exp. Med.*, 1926, xliii, 573) and in others to follow.

TABLE II.

*Reaction of Rabbits to an **Intranasal** Instillation of Bact. lepisepticum, Rivers Type D.*

Rabbit No.	Condition before inoculation.		Duration of life after inoculation.	Cultures of heart's blood, lungs, nasal passages.	Type of pneumonia.			Location of bacteria.	Orientation of exudate.	Type of exudate.	Blood vessels and lymphatics.	Bronchi.	Survivors.	
	Snuffles.	Carriers.			Acute diffuse.	Lobar distribution.	Chronic pleuropneumonia.						Snuffles.	Carriers.
			days											
D88 I*	0	0	2	+	++			Subpleural.	Subpleural; perivascular.	Fibrin, eosinophils, few mononuclears.	+	0	–	–
D36 I	0	0	2	+	++			"	" "	" "	+	0	–	–
D43 I	0	0	2	+	++			Perivascular.	Perivascular.	" "	++	0	–	–
D54 G†	0	0	2	+	++			" some subpleural.	" some subpleural.	" "	++	0	–	–
C79 G	0	0	2	+	++			Subpleural.	Subpleural.	" "	+	0	–	–
C83 G	+	+	3	+	++			" perivascular.	" perivascular.	" "	++	0	–	–
C95 G	0	+	3	+	No change.								–	–
D69 I	0	0	6	+		++							–	–
D92 I	0	0	9	+		++							–	–
D70 I	0	0	9	+		++			Intraalveolar.	Eosinophils, mononuclears, epithelial cells.	+	+	–	–
D74 I	0	0	10	+			++						–	–

| | | |

blood broth culture was used, and the dosage in numbers of bacteria was estimated by plating methods. Certain rabbits were born and bred in the Rockefeller Institute breeding room, which has been entirely free of pneumonias, snuffles, and *Bact. lepisepticum* carriers for 2 years. These have been specially designated in the protocols. Preliminary routine nasal swabs from rabbits used in the experiments showed no pathogenic organisms. The animals weighed usually 1000 to 1200 gm. each.

Experiment 1.—Only twelve rabbits, bred at the Rockefeller Institute, were available for this experiment, so the total number was increased to twenty-one by adding nine more from the general stock.[2] Each animal was placed in a separate cage and cultured three times for the presence of *Bact. lepisepticum* in its nasal passages. None of the Institute animals proved to be carriers; two of the general stock showed signs of clinical snuffles; these and two others were found to be carriers. 2 weeks later all were given intranasally ½ cc. (500,000,000 bacteria) of a 17 hour blood broth culture of Rivers strain, Type D. Daily observations were continued, and the animals which died were autopsied, cultured, and the lungs sectioned for histological study.

In this experiment, the general stock animals behaved in the same manner as the specially bred rabbits. The results charted in Table II are the following: First, the rabbits differed in their response to a uniform dose of the same culture of *Bact. lepisepticum* introduced by way of the nares, the normal portal of entry. Eleven died of pneumonia 2 to 12 days after inoculation; three survivors developed chronic snuffles, and four remained clinically well. Second, the lung changes found at autopsy were quite indistinguishable from many of the spontaneous cases described above (Fig. 1). Third, the pneumonias fell into three general groups, according to duration of life.

Six animals died within 3 days, and at autopsy showed moist, red lungs, diffusely engorged (Fig. 2, *a*). Under the microscope, sections stained by Giemsa's method showed clumps of bacteria, fibrin, and eosinophils just beneath the pleural endothelium, in the pleural, subpleural, and interlobular draining lymphatics, and in the perivascular lymphatics (Fig. 2, *c*). Similar masses of bacteria and exudate filled many of the smaller blood vessels (Fig. 2, *b*). Eosinophils were abundant in the blood stream, and perivascular and interstitial tissue. The interstitial frame-

[2] Rabbits for the general stock are ordinarily purchased from outside dealers.

work, especially in the region of the smaller veins and arteries, was distended by swollen capillaries, leucocytes, serum, and scattered bacteria. Many alveoli contained serum. Subpleural hemorrhages were not infrequent. The blood vessels contained many polymorphonuclear leucocytes, some of which were migrating between the cells of the vessel wall. The bronchi seemed quite normal.

Three rabbits survived from 6 to 9 days, and showed, at autopsy, an extensive lobar type of pulmonary consolidation, with or without fibrinous pleurisy (Figs. 1 and 3, *a*). The sections showed the normal architecture of alveoli, obliterated by solid masses of epithelial cells, some of which were in active stages of multiplication (3, *b*). There was some subpleural exudate. No bacteria were seen. The lymph

TABLE III.

Incidence of Different Types of Pneumonia Following Intranasal Instillation of Bact. lepisepticum.

Experiment.	Total No. of rabbits	Total No. dying of pneumonia.	Red, moist lungs; exudate interstitial, with subpleural and perivascular orientation.	Large, grey lungs, rubbery; lobar consolidation.	Pleuropneumonia.
Oct. 26, 1923	5	2	1	1	0
Nov. 5, 1923	12	3	1	1	1
Feb. 13, 1924	33	4	3	0	1
" 17, 1924	10	3	1	1	1
Apr. 21, 1924	10	2	0	2	0
May 2, 1924	9	3	0	0	3
Feb. 13, 1925	7	1	0	0	1
Apr. 14, 1925	16	4	3	1	
Total	102	22	9	6	7
Per cent	100	22	41	27.2	31.8

sinuses were swollen; blood vessels contained many eosinophilic cells. In some instances the bronchi were filled with loose masses of leucocytes and débris. The epithelium lining these bronchi seemed normal.

Two rabbits survived 10 to 12 days, and presented, at autopsy, collapsed, grey, shaggy lungs in chest cavities filled with purulent fluid and lined by fibrinous exudate.

Still other experiments indicated that the pneumonias might be grouped in the same way (Table III). In each instance animals dying within 2 or 3 days after inoculation showed accumulations of

bacteria, fibrin, red cells, and eosinophils, in and about smaller blood vessels and lymphatics, and diffuse, interstitial engorgement and leucocytic exudate, while the more chronic cases displayed either the large, rubbery lungs with lobar type of consolidation (Fig. 1), or thoracic cavities filled with purulent fluid and containing collapsed, grey lungs.

Since all the rabbits of each experiment were injected with a like dose of the same strain of organisms, it is evident that the different types of pneumonia which followed were due entirely to variations in the resistance of the animals employed.

The manner of entry of the bacteria into the lungs is not directly revealed in the experiments described. However, the animals showing the acute, diffuse type of pneumonic reaction presented histological appearances suggesting that the bacteria reached the lungs by way of the blood or lymph stream rather than directly through the bronchi. To test this point, the next series of experiments was carried out.

Intravenous, Intratesticular, and Subcutaneous Inoculations.

It seemed probable that if the pneumonias arising spontaneously, or induced by the nasal instillation of *Bact. lepisepticum*, were incited by the organisms invading the lungs by the blood or lymph, these effects could be reproduced by introducing the organisms into a vein or vascular organ; and, on the contrary, if the portal of entry was by way of the trachea and bronchi, intratracheal inoculation should more nearly duplicate the conditions observed. In the following experiments bacteria were introduced intravenously, intratesticularly, and subcutaneously, and the resulting pulmonary lesions were compared with the spontaneous and nasal inoculation pneumonias.

Experiment 2.—½ cc. (500,000,000 bacteria) of a 17 hour rabbit blood broth culture of *Bact. lepisepticum*, Rivers strain, Type D, was given to twenty-four stock rabbits. Eight received the culture into the ear veins, eight intratesticularly, and eight subcutaneously in the lumbodorsal region. They were then placed in individual cages and observed for 2 months. Dead animals were autopsied, cultured, and the lungs taken for histological study.

Three of the eight animals injected into the ear vein were dead within 17 hours. Blood and lung cultures were positive, and the

lungs showed intense engorgement of all capillaries and small blood vessels. The distended interstitial tissue contained a few eosinophils. Serum filled many alveoli. Leucocytes were scattered near the larger blood vessels. In the other five animals, blood cultures were positive 30 hours after inoculation; at 48 hours two were dead and showed smaller lesions. On the 3rd and 5th days the remaining three died; the lesions of one were identical with those of the other cases, and two showed pleuropneumonia and pericarditis.

One of the eight rabbits injected subcutaneously died after 48 hours and showed an acute, diffuse pneumonia with positive blood and lung cultures; three others showed positive blood cultures at this time. On the 5th day two more died and presented the same type of pneumonia; on the 6th day two more succumbed and showed extensive pleuropneumonia. The remaining three animals survived and developed chronic abscesses at the site of inoculation.

The eight rabbits injected intratesticularly were dead within 17 hours. Heart's blood and lung cultures were positive. Hemorrhages were noted in the thymus and testicle. The lungs showed extensive, early changes, consisting of engorged capillaries, serum, red blood cells, and a few leucocytes distending the interstitial tissue. In four cases this exudate was most prominent beneath the pleura, where bacteria were also seen. Perivascular lymphatics containing eosinophils and mononuclears stood out prominently.

We may summarize the results of this type of experiment by stating that *Bact. lepisepticum*, when introduced into the blood directly, or by way of the testicle, tends to produce severe congestion of the lungs, accompanied by an outpouring of serum into the alveoli and interstitial tissue. However, in certain animals, which survive longer, the bacteria assemble beneath the pleura and in the perivascular lymphatics, and in and about the capillaries and small veins, in which cases, leucocytes collect in the interstitial tissue and fill many of the alveoli. These lesions are indistinguishable from those met with in the spontaneous pneumonia and in the pneumonia incited by the intranasal instillations.

When the bacteria were injected subcutaneously, the lungs showed more chronic changes, and the bacteria and exudate were especially prominent beneath the pleura, around the capillaries, small veins, and

in the perivascular lymphatics. These cases were similar to the spontaneous and intranasal lobar and pleural pneumonias.

We conclude, therefore, that *Bact. lepisepticum*, reaching the lungs by way of the blood and lymph, induces pneumonias indistinguishable from those occurring so called spontaneously, or following intranasal instillation.

Intratracheal Inoculation.

Although typical pneumonias may follow the hematogenous distribution of *Bact. lepisepticum*, one cannot ignore the possibility of the aerogenous route of entry of the bacilli. Indeed, many spontaneous cases in the chronic pleuropneumonia group showed, on section, bronchial walls involved in an inflammatory process and lumina filled with pus, and the same process was also noted in an occasional chronic pleuropneumonia case following intranasal instillation of bacteria. So the question arises whether the primary lung infection is, after all, not hematogenous, and the local extension by way of the bronchi, or whether the initial lesion is aerogenous and the subsequent invasion and spread take place by way of the blood stream. The following experiments were planned to answer these questions.

Experiment 3.—Seventeen stock rabbits, weighing 2000 to 2300 gm. each, with one exception, were placed in separate cages and injected with a 17 hour blood broth culture of *Bact. lepisepticum*, Rivers strain, Type D. A dose of 300,000,000 organisms in 2 cc. of broth was given intratracheally to eleven rabbits by means of a soft urethral catheter inserted to the bifurcation. The animals were anesthetized lightly, so that the bacteria could be instilled without trauma.[3] At the same time, the six remaining rabbits were given a like dose intratesticularly, in order to control the degree of virulence of the organisms and to be sure that the effects of the intratracheal insufflations were due entirely to host factors and not to altered pathogenicity of the microbes. Temperatures and blood cultures were taken, and the dead animals were autopsied, cultured, and the organs sectioned for histological study.

The results of this experiment are summarized briefly as follows

[3] Intratracheal punctures with a hypodermic needle invariably resulted in localized reactions extending by way of the subepithelial tracheal tissues, directly into the chest cavity, septicemia, acute, diffuse pneumonia, and death.[14]

(Table IV): Five of the six animals injected intratesticularly were dead within 8 hours. Blood cultures taken from ear veins 3 hours after inoculation were positive. Very little change was noted in lungs or other organs at autopsy. Heart's blood smears, examined within $\frac{1}{2}$ hour after death, showed bacteria outnumbering red cells about 50 to 1. One rabbit, weighing 3500 gm., survived, although septicemia continued for more than 17 hours. Six of the intratracheal rabbits died of pneumonia and septicemia; four had shown positive blood cultures within 17 hours after inoculation. Of these, two died within 48 hours, one of a typical, acute, diffuse pneumonia with subpleural orientation, and one with a large, consolidated area at the hilum, extending toward the periphery. This region was surrounded by a collar of bacteria, red blood cells, fibrin, and eosinophils. Within it, the lung architecture was distorted, alveolar walls were partly destroyed, cell nuclei stained poorly. Plugs of cell débris, fibrin, and serum, mixed with bacteria, filled the alveoli and bronchi. The walls of the latter were edematous and contained numbers of bacteria and leucocytes, tangled in fibrin which seemed to pour through the epithelial membrane at regular intervals. Three more died on the 4th day, two having large, well defined areas at the hilum, and all three showing some diffuse, subpleural involvement. One other rabbit died on the 5th day, showing in one lung the lesion at the hilum, and in the other diffuse, subpleural lesions. Five animals which had shown no positive blood cultures survived.

By repeating this kind of experiment, several facts became apparent. First, bacteria introduced into the lungs by way of the bronchi induce pneumonia with a frequency of not more than 60 per cent. Second, this pneumonia is relatively chronic. Third, the primary lesion is at the hilum and spreads by direct extension. Fourth, the cases of diffuse, subpleural pneumonia characteristic of blood invasion which occur are due possibly to artificial trauma, but more probably, since bacteriemia could usually be demonstrated within 24 hours in all cases, to invasion of the blood stream at the site of the primary hilum lesion.

A final series of experiments was carried out to study very early lesions following intratracheal inoculations.

TABLE IV.

Response of Rabbits to Intratracheal and Intratesticular Inoculation of Bact. lepisepticum.

Rabbit No.	Site of inoculation.	Hrs. and days after inoculation.																Duration of life.	Heart's blood and lung cultures.	Type of pneumonia and remarks.
		3 hrs.		9 hrs.		13 hrs.		17 hrs.		26 hrs.		48 hrs.		4 days.		6 days.				
		Blood culture.	Temperature.	Blood culture.	Temperature.	Blood culture.	Temperature.	Blood culture.	Temperature.	Bood culture.	Temperature.	Blood culture.	Temperature.	Blood culture.	Temperature.	Blood culture.	Temperature.			
			°F.		°F.		°F.		°F.		°F.		°F.		°F.		°F.	*hrs.*		
E71	Intratesticularly.	+																4	+++	Very little change in lungs or organs.
E61	"	+																5.5	+++	" "
E96	"	+																5.75	+++	" "
E47	"	+																7	+++	" "
E13	"	+																8	+++	" "
E66	"	+		+		0		+		0		0	104.1		104.2		104.1	S.		Abscess in testicle.
E73	Intratracheally.	+		+		0		+		+								48	+	Large lungs, hemorrhagic; lesion at hilum.
E59	"	0		0		0		+		+								48	+	Large, hemorrhagic lungs. Diffuse subpleural lesions.
																		days		
E70	"	+		0		0		0		+		+	103.8					4	+	Pleuropneumonia. Lesion at hilum.
E78	"	0		0		0		+		+		0	104.1					4	+	Pleuropneumonia. Diffuse subpleural lesion.

E81	"	0	0	0	0	0	0	106.3			4	+	Pleuropneumonia. Lesion at hilum.
E67	"	0	0	0	0	0	0	104.2	100.1		5	+	Pleuropneumonia. One lung with lesion at hilum; other lung with diffuse subpleural lesions.
5	"	0	0	0	0	0	0	103.3	102.9	100.9	S.		
E95	"	0	0	0	0	0	0	102.4	102.8	101.1	"		
E57	"	0	–	0	0	0	0	102.6	101.6	103.3	"		
D04	"	0	0	0	0	0	0	103.1	102.7	102.4	"		
E32	"	0	0	0	0	0	0	103.2			"		Diarrhea.

Experiment 4.—Nine rabbits from the general stock, with no *Bact. lepisepticum* in their nasal passages, and no pulmonary lesions on x-ray examination, were anesthetized lightly and injected intratracheally by means of a soft catheter with a similar dose of the same organisms used in the previous experiment. X-rays, blood cultures, and temperatures were taken at irregular intervals, according to clinical symptoms.

4 hours after injection, all temperatures were between 101.2° and 103.7°; blood cultures and x-rays of the lungs were negative. At 24 hours one rabbit, with temperature of 106.3° and suspicious x-ray, was killed and found to have a massive grey consolidation of the entire right middle lobe. Cultures from the heart's blood were positive. Two other animals with normal temperatures and questionable x-ray plates were also killed, but showed no lesions. On the 3rd day two animals had temperatures of 105.5° and 105.9° and x-ray shadows in the right lungs. Blood cultures were positive. These animals at autopsy showed grey consolidation of the entire right middle and lower lobes. The remaining four animals manifested no change whatsoever during a 1 month period of examination.

The histological examination of tissues from the infected animals showed at the hilum, either a massive lesion, or a number of smaller foci about the main bronchi (Fig. 4, *a*). The walls of these bronchi were edematous and filled with leucocytes; the epithelial lining was sloughing and invaded with polymorphonuclear eosinophils, and at intervals, as though through pores, an exudate was spreading out over the surface into the lumen (Fig. 4, *b* and *c*). All about were alveoli filled solidly with leucocytes and fibrin, and in each case, sharply demarcating the infected area from normal lung tissue, were band-like accumulations of bacteria, fibrin, and leucocytes in various stages of necrosis.

Evidently, therefore, large doses of *Bact. lepisepticum*, reaching the lungs by way of the air tubes, induce pneumonia in about 50 per cent of the animals so treated. A relatively chronic infection develops at the base of the lung and spreads locally by direct extension, and generally by way of the blood stream. This process, which, in its early stages, is easily distinguishable from pneumonias induced experimentally by putting organisms into the blood stream, and from the acute, diffuse spontaneous pueumonias, may continue for several days and finally involve both lungs, pleura, and pericardium.

DISCUSSION.

That the intranasal instillation of cultures of *Bact. lepisepticum* will induce pneumonia in rabbits has been pointed out by Smith, Beck, Volk, Davis, Saelhof, and probably others.[4] From the epidemiological point of view, this class of experiments takes on a new interest, when viewed in the light of reciprocal action of parasite and host. We have asked ourselves what it is that determines whether an animal resists an otherwise infective dose of the culture, or develops a localized infection, or succumbs to a pneumonia attended by septicemia.

Wadsworth emphasized the host's resistance as represented by the response of living tissues to the inoculation of pneumococci in his experiments on pneumococcus infection in the rabbit.[5] He writes: "Lobar pneumonia is simply one phase of pneumococcus infection—it was not wholly the bacterial incitant, but the susceptibility of the host as well, that determined whether the disease processes would be bronchopneumonic, lobar, or bacteriemic[6]—the extent of lung lesion is simply an expression of the resistance of the individual." Lamar and Meltzer, on the other hand, disregarded the host's resistance and ascribed the effects wholly to the pathogenic microorganism, as is shown by their statement that "the causation of the pneumonia and its outcome depended entirely upon the invading organism, its numbers and virulence; and the condition of the host was an inconsiderable factor in the result."[7] However, in the more recent investigations of Winternitz, Sisson and Walker, Cecil and Blake, Permar, and Stillman and Branch, the influence of the host is more or less stressed.

The pathological findings described have led us to set up the following classes for the spontaneous pneumonic processes observed: (1) acute, diffuse, or septicemic; (2) lobar; (3) chronic pleural; and (4) abscessive. It is interesting to have ascertained that the first three classes or types of disease can be reproduced experimentally by employing cultures of sufficient virulence, and when rabbits which have

[4] Smith, T., *J. Comp. Med. and Surg.*, 1887, viii, 24. Beck, M., *Z. Hyg. u. Infectionskrankh.*, 1893, xv, 362. Volk, R., *Centr. Bakt., 1. Abt., Orig.*, 1902, xxxi, 177. Davis, D. J., *J. Infect. Dis.*, 1913, xii, 42; 1917, xxi, 314. Saelhof, C. C., 1921, xxviii, 374.

[5] Wadsworth, A. B., *Am. J. Med. Sc.*, 1904, cxxvii, 851.

[6] Wadsworth, A. B., *J. Exp. Med.*, 1912, xvi, 54.

[7] Lamar, R. V., and Meltzer, S. J., *J. Exp. Med.*, 1912, xv, 133.

been especially bred and protected from exposure to *lepisepticum* infection are employed for intranasal inoculation. The incidence of each type of infection is determined by host factors. Thus, on the basis of our experiments, we may state that chronic pleuro-pneumonia arises when the rabbits are moderately resistant, lobar pneumonia when the animals are still somewhat less resistant, and diffuse pneumonia and septicemia when the resistance is least marked.

The manner of entry, portal of entry, so called, of the bacteria into the lungs, after intranasal instillation, is important. Two views are generally held; namely, that infection occurs by way of the air and by way of the blood. For the most part, experiments dealing with this question have been made with microorganisms of human origin introduced into rabbits. The present undecided state of our knowledge of this subject is shown by the following brief review.

Kidd,[8] reviewing the literature in 1912, stated that although investigators had been unable to induce lobar pneumonia in animals by the intravenous injection of pneumococci, clinical evidence favored its hematogenous origin in man. Wadsworth,[5,6] Lamar and Meltzer,[7] Permar,[9] Armstrong,[10] Blake and Cecil,[11] Stillman and Branch,[12] and Gaskell[13] obtained pneumonias in animals only by a route apparently aerogenous. Winternitz, Smith, and Robinson,[14] however, considered that some of these methods led to infection of the tracheal tissue which was, in turn, followed by a lymphogenous or hematogenous pneumonia.

Saelhof[4] studied *lepisepticum* pneumonia in the rabbit, and concluded that the organism entered the lungs by way of the blood stream.

Our experiments confirm the view that infection occurs usually by way of the blood stream. We induced pneumonic conditions resembling the spontaneously occurring ones, as well as those arising after nasal instillation of cultures, by direct blood stream inoculations.

Support in the present connection is found in the results of intra-

[8] Kidd, P., *Lancet*, 1912, i, 1589.

[9] Permar, H. H., *J. Med. Research*, 1923–24, xliv, 1.

[10] Armstrong, R. R., *Brit. Med. J.*, 1914, ii, suppl., 57.

[11] Blake, F. G., and Cecil, R. L., *J. Exp. Med.*, 1920, xxxi, 403, 445, 499.

[12] Stillman, E. G., and Branch, H. A., *J. Exp. Med.*, 1923–24, xxxviii and xl.

[13] Gaskell, J. F., *J. Path. and Bact.*, 1925, xxviii, 427.

[14] Winternitz, M. C., Smith, G. H., and Robinson, E. S., *Bull. Johns Hopkins Hosp.*, 1920, xxxi, 63.

tracheal insufflation of cultures, which are either without effect, or, infection ensuing, lead to a chronic pneumonic process, of primary, peribronchial, basal form, and of slow progression.

The application of these findings to occurrences in man is entirely analogical. And yet, there are many observations upon human beings which fall in with the views regarding mode of infection expressed in this paper and based on the experiments described.

CONCLUSIONS.

1. Four general types of spontaneous pneumonia, associated with strains of *Bact. lepisepticum* similar in biology and virulence, are described: (1) an acute, diffuse form, with subpleural and perivascular orientation of the exudate, (2) lobar, (3) pleuro-, and (4) abscess pneumonia.

2. The acute, diffuse, lobar, and pleuro pneumonias may be induced experimentally by intranasal instillation of a virulent strain of *Bact. lepisepticum*.

3. These same types occur when the organisms are inoculated intravenously, intratesticularly, and subcutaneously.

4. Intrabronchial insufflation of the organisms brings about infection in less than half of the animals. When effective, a sharply circumscribed, peribronchial lesion is found at the base of the lung, which spreads peripherally by direct extension, and generally by invasion of the blood stream.

5. It is concluded that differences in the types of pneumonia following infection with similar strains of *Bact. lepisepticum* depend upon the resistance of the animal, and that the usual portal of entry of this organism into the lungs, in cases of acute and lobar pneumonia, is by way of the blood stream.

EXPLANATION OF PLATES.

PLATE 20.

FIG. 1. Experimental rabbit pneumonia following nasal instillation of *Bact. lepisepticum*. Lobar consolidation.

PLATE 21.

FIG. 2, *a* to *c*. Acute diffuse pneumonia; intranasal instillation of Rivers D strain into a susceptible rabbit.

(*a*) Cross-section of rabbit's lung showing subpleural and perivascular distribution of exudate. × 6.

(*b*) Normal bronchus and small blood vessel surrounded by eosinophils and mononuclears. Nearby are similar accumulations in the interstitial spaces. × 140.

(*c*) Photomicrograph of lung surface to show subpleural orientation of fibrin, red cells, and eosinophils. × 460.

PLATE 22.

FIG. 3, *a* and *b*. Lobar type of pneumonia; intranasal instillation of Rivers D strain into a more resistant host.

(*a*) Cross-section of rabbit's lung showing consolidation of entire organ. × 6.

(*b*) Photomicrograph of collapsed alveoli, proliferating alveolar cells, and accumulations of mononuclears. × 650.

PLATE 23.

FIG. 4, *a* to *c*. Basal type of pneumonia; intratracheal instillation of Rivers D strain into a relatively susceptible animal.

(*a*) Cross-section of lung showing localization of exudate at hilum. × 6.

(*b*) Photomicrograph of bronchial wall with polymorphs, eosinophils, and mononuclears going between the alveolar epithelium at regular intervals toward the masses of bacteria which lie in the lumen. The bronchial wall is above; the lumen, filled with exudate, below. × 430.

(*c*) A different bronchus showing a similar process at a higher magnification (× 1000). Here the bronchial lumen is above, and the bronchial wall below.

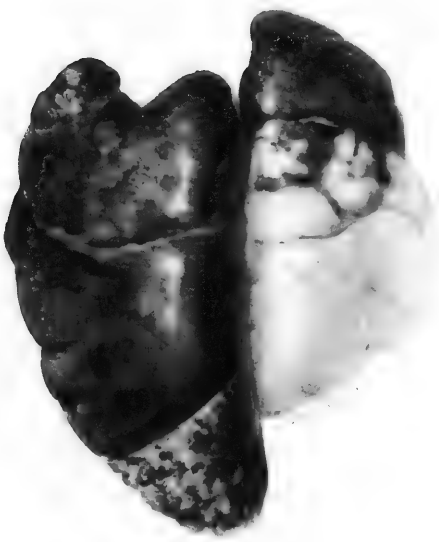

FIG. 1.

(Webster: Respiratory infections in rabbits. VII.)

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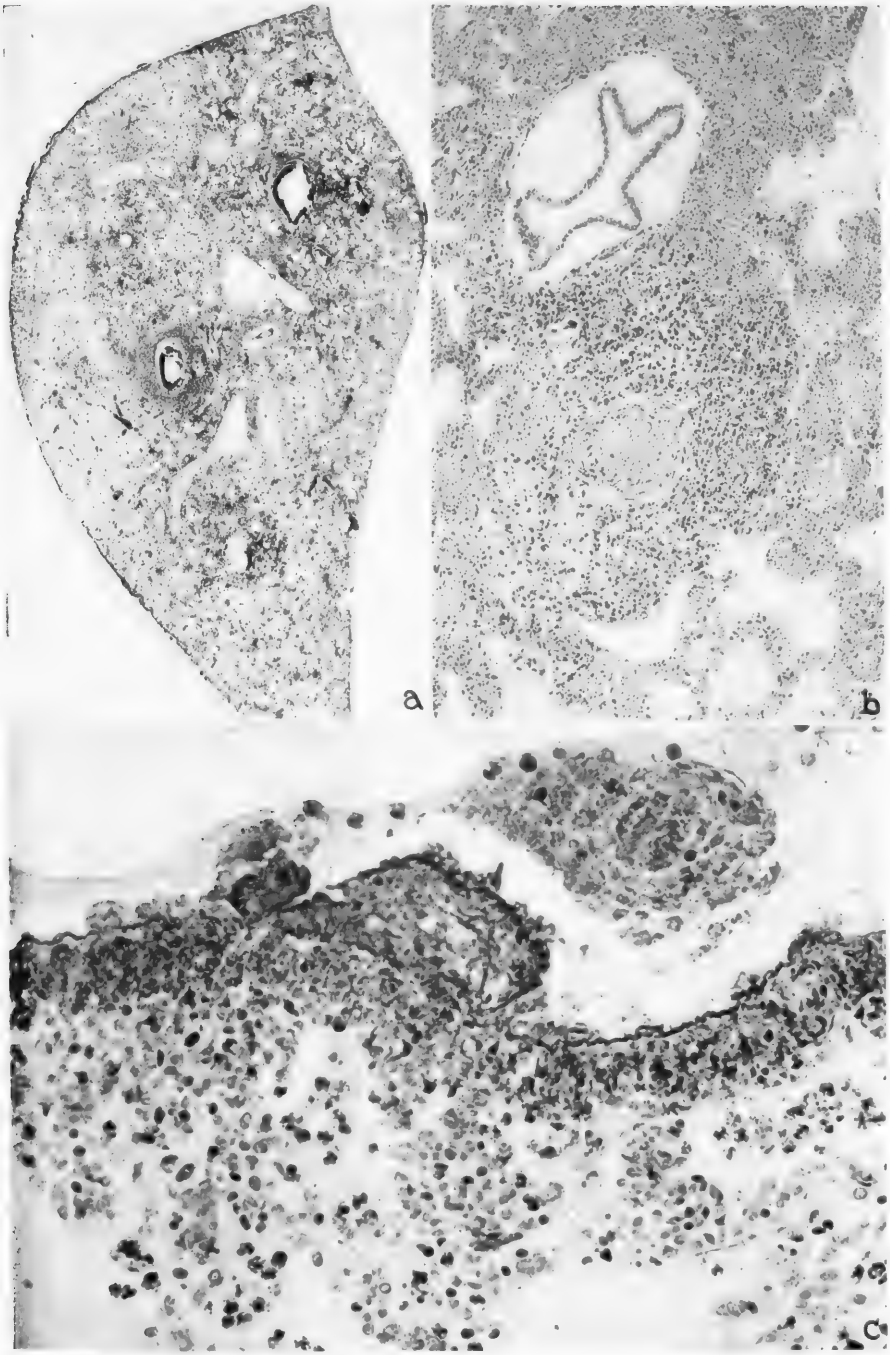

FIG. 2.

(Webster: Respiratory infections in rabbits. VII.)

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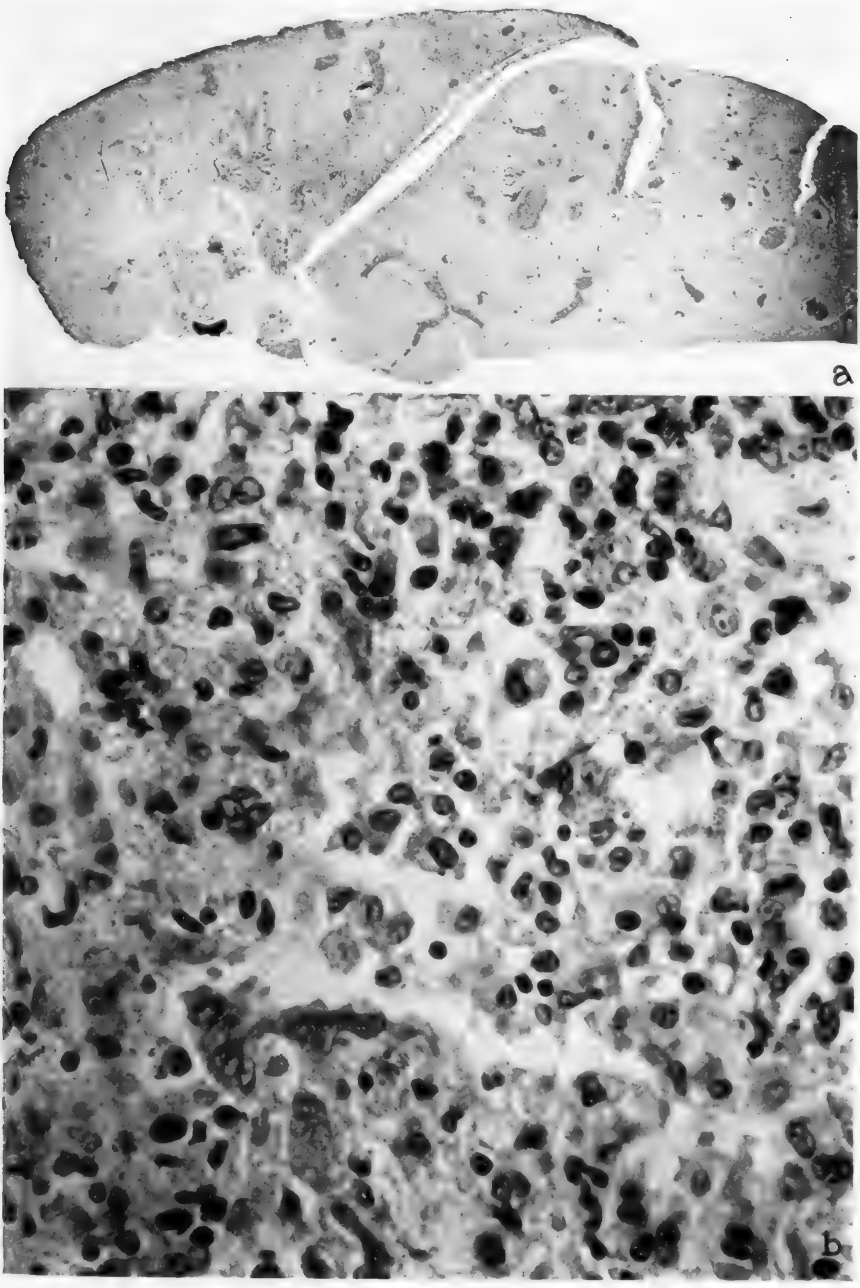

FIG. 3.

(Webster: Respiratory infections in rabbits. VII.)

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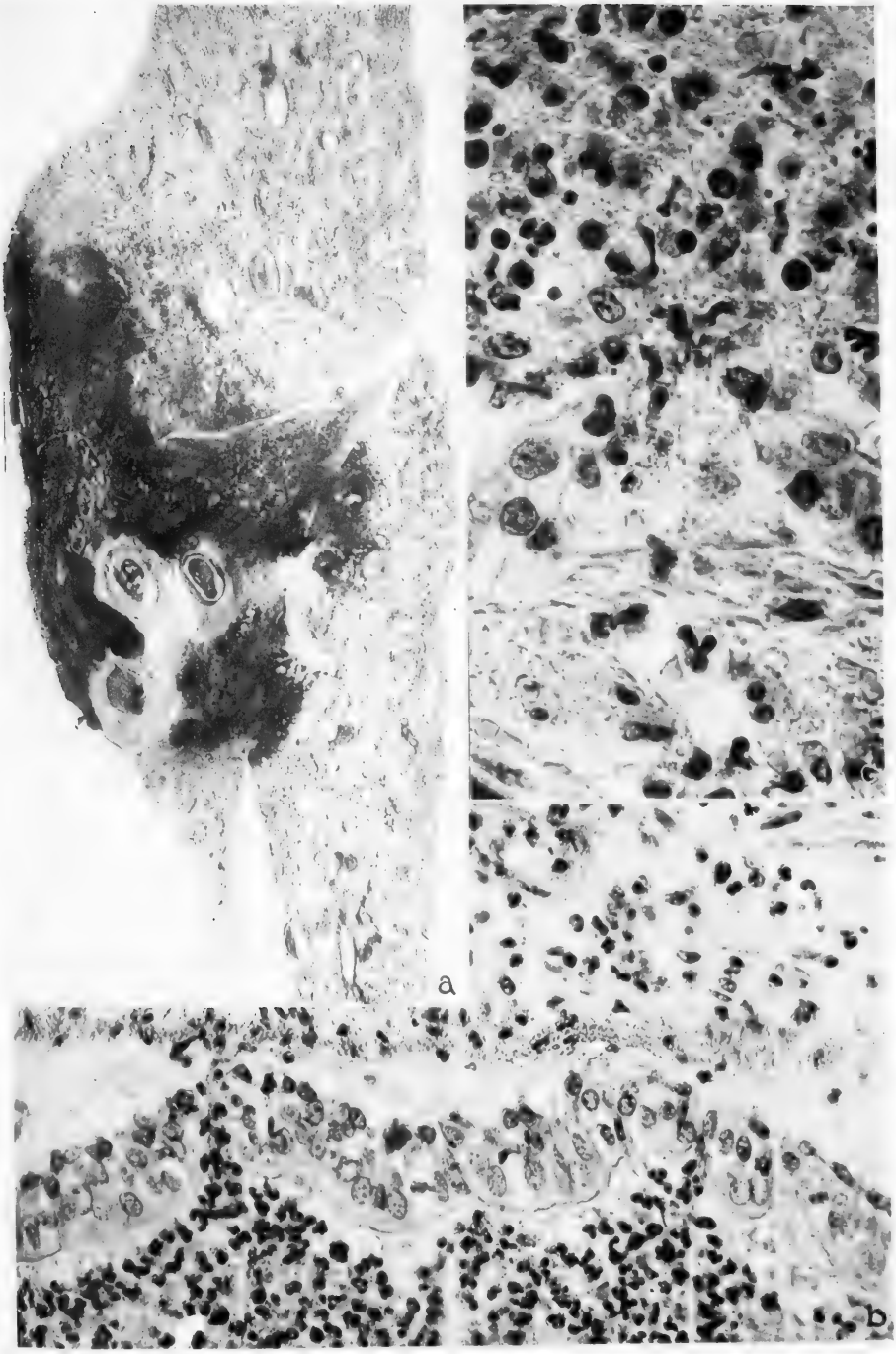

FIG. 4.

(Webster: Respiratory infections in rabbits. VII.)

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EPIDEMIOLOGICAL STUDIES ON RESPIRATORY INFECTIONS OF THE RABBIT.

VIII. Carriers of Bacterium lepisepticum.

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Plates 24 and 25.

(Received for publication, January 8, 1926.)

That carriers of *Bacterium lepisepticum* occur in laboratory rabbits and other stocks is of course well known. We have dealt with certain aspects of this matter in earlier papers of this series. Furthermore, as we have pointed out, similar chronic carriers of this organism may be developed experimentally by instilling cultures into the nares of specially bred rabbits which have been protected from previous exposure. The fact that an enduring carrier state can be brought about in this manner affords opportunity for studying it under controlled conditions, and for analyzing the factors necessary for this particular state of balance or equilibrium between parasite and host.

The present paper deals with experiments which show that certain types of *Bact. lepisepticum* are capable of prolonged vegetation in the mucous membranes of rabbits, while others are not. The nasal passages of the animals used in these experiments were examined microscopically, in order to determine the nature and severity of the tissue reactions, and to correlate them with the bacteriological findings. We have considered the results of the experiments in the light of our other investigations of this infection; and finally have endeavored to summarize the factors which condition the response of the rabbit host to the presence of the parasite, *Bact. lepisepticum*.

Method.

Groups of 800 to 1000 gm. rabbits, protected from any exposure to *Bact. lepisepticum*, were taken from the Rockefeller Institute breeding room, placed in

separate cages, and given, intranasally, by means of a blunt syringe, rabbit blood broth cultures of various strains of *Bact. lepisepticum* and *B. bronchisepticus*. Subsequently, each animal was subjected to close observation, temperature was recorded frequently, and the nasal passages were cultured at intervals over a period of several weeks. Material was taken from the nares by inserting a sterile cotton swab, to a distance of 2 to 3 cm. into the passage, and then spreading it over hemolyzed rabbit blood agar in 15 cm. Petri dishes. After 24 to 48 hours incubation, the colonies on the plates were examined qualitatively and quantitatively. Rabbits which lived until the end of the experiment were killed by injecting 5 cc. of air into the ear vein. These and the other animals which died of infection were autopsied, cultured, and their nasal passages sectioned for further study. The entire premaxillary, maxillary, and nasal portion of the skull was removed in one piece, decalcified, and divided, through the nasal passages, into three blocks: anterior, medial, and posterior. Sections from these blocks were stained by Giemsa's method and with hematoxylin and eosin.

At four separate times, from April, 1924, to July, 1925, groups of rabbits were treated in this way, and as controls two more uninoculated series of eleven and thirty similar rabbits were cultured, autopsied, and their nasal tissues sectioned.

The following bacterial cultures were used in the experiments: six strains of *Bact. lepisepticum* and four of *B. bronchisepticus*.

The Rivers and No. 103 D strains of *Bact. lepisepticum* are antigenically identical with De Kruif's cultures, and show the same high degree of virulence. They were obtained from rabbits at the Rockefeller Institute, which had died of the natural infection. But these D strains, so common in this laboratory from 1921 to 1923, have not been recovered by us from these or other rabbits since that time.

The G variant of the Rivers D strain is similar to the avirulent G mutants described by De Kruif.[1] It was obtained by growing the original D culture in plain broth at atmospheric oxygen pressure for 72 hours.

The mucoid strains form large, fluorescent, flowing colonies, are culturally similar to the D strains, but show definite serological differences; they are less virulent, and are at present widespread throughout the Rockefeller Institute stock and at least two other rabbit populations which we have under observation. Strains 329 and 544 were obtained from the nasal passages of "healthy" rabbits in this laboratory. Strain DC 30 was obtained from a rabbit dead of pneumonia at a rabbit farm in New City, N. Y.

Two strains of *B. bronchisepticus*, Nos. 8 and 188, were obtained from the nasal passages of "healthy" rabbits. No. 9 was recovered from the lung of a dog dead of distemper, and B from a rabbit epidemic. Culturally, they are all alike and their virulence for guinea pigs, on intranasal inoculation, is similar. They induce little or no gross infection in rabbits, when given by the normal, nasal portal of entry, and may be recovered from the nasal passages of a large percentage of the rabbits in the communities which we have been studying.

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[1] De Kruif, P. H., *J. Exp. Med.*, 1921, xxxiii, 773; 1922, xxxvi, 309.

EXPERIMENTAL.

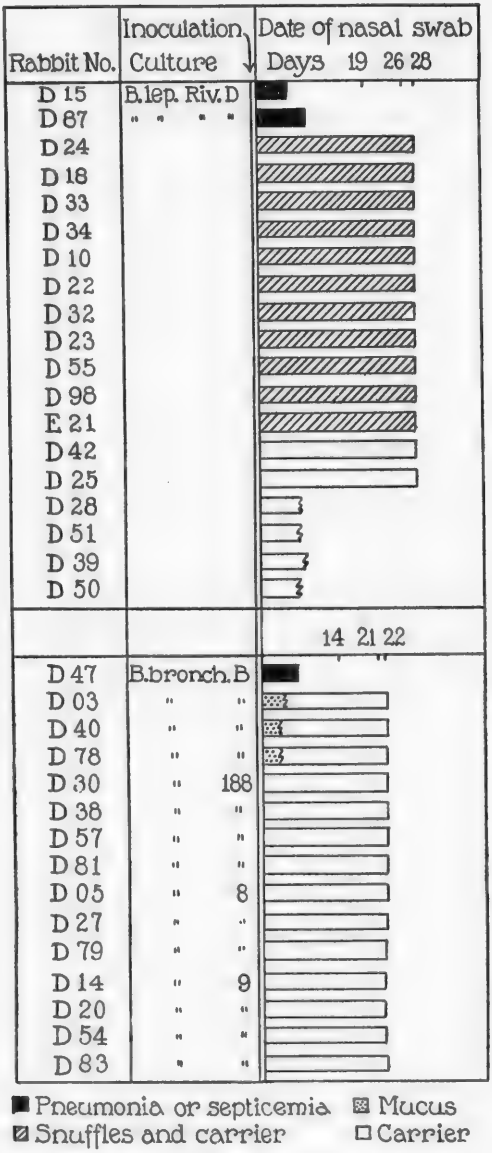
The technique used in each of the following four experiments is similar, and the purpose of each is to determine what types of bacteria and what conditions of the host lead to the equilibrium known as the "healthy" carrier state. For the sake of clarity, the results have been grouped as follows: those referable to the bacteria used in each test are included with the description of the particular experiment, while the reactions of the host, especially the changes which take place in the nasal tissues as a result of the presence of these bacteria, are considered together in a special paragraph.

Experiment 1.—April 21, 1924, nineteen rabbits were given into each naris ½ cc. (250,000,000 bacteria), of a 17 hour blood broth culture of *Bact. lepisepticum*, the virulent Rivers D strain. 5 days later, fifteen similar rabbits were given intranasally different cultures of *B. bronchisepticus*, 1,000,000,000 bacteria to each animal. Strains 188, 9, and B were administered to four animals each, and Strain 8 to three.

4 weeks later the survivors, together with eleven control animals, were killed, cultured, and their nasal passages sectioned for further study.

The clinical and bacteriological results of this experiment are summarized in Text-fig. 1. Of the nineteen animals given the Rivers D strain of *Bact. lepisepticum*, two died of pneumonia, eleven developed chronic snuffles, two became "healthy" carriers, and four proved entirely refractory to infection. Type G variants[1] appeared in the nasal passages of three of the animals with chronic snuffles and seemed to increase in number at each examination (Table I). Fourteen of the fifteen rabbits receiving the cultures of *B. bronchisepticus* became "healthy" carriers. One animal died of pneumonia.

Of chief interest to us at this time is the striking difference between the "healthy" carrier rate of the *Bact. lepisepticum*, Rivers D strain group—10.5 per cent—and that of the *B. bronchisepticus* groups—93.5 per cent. Apparently, this difference is due to bacterial rather than host factors, since dosage and host variables were adequately controlled. We conclude, therefore, that (1) these virulent types of *Bact. lepisepticum*, which are not found at present in the respiratory passages of our "healthy" stock rabbits, are likewise unable to survive there when instilled artificially, but that (2) strains of *B. bronchisepticus*, which are less virulent and which do vegetate naturally in the nasal tissues of a large percentage of our so called normal rabbits,



TEXT-FIG. 1. Response of specially bred rabbits to intranasal inoculation of *Bact. lepisepticum* and B. *bronchisepticus*. Experiment 1.

persist there equally well and indefinitely when introduced experimentally.

TABLE I.

Appearance of Type G Variants in Nasal Passages of Rabbits Inoculated with Bact. lepisepticum, Rivers Strain, Type D.

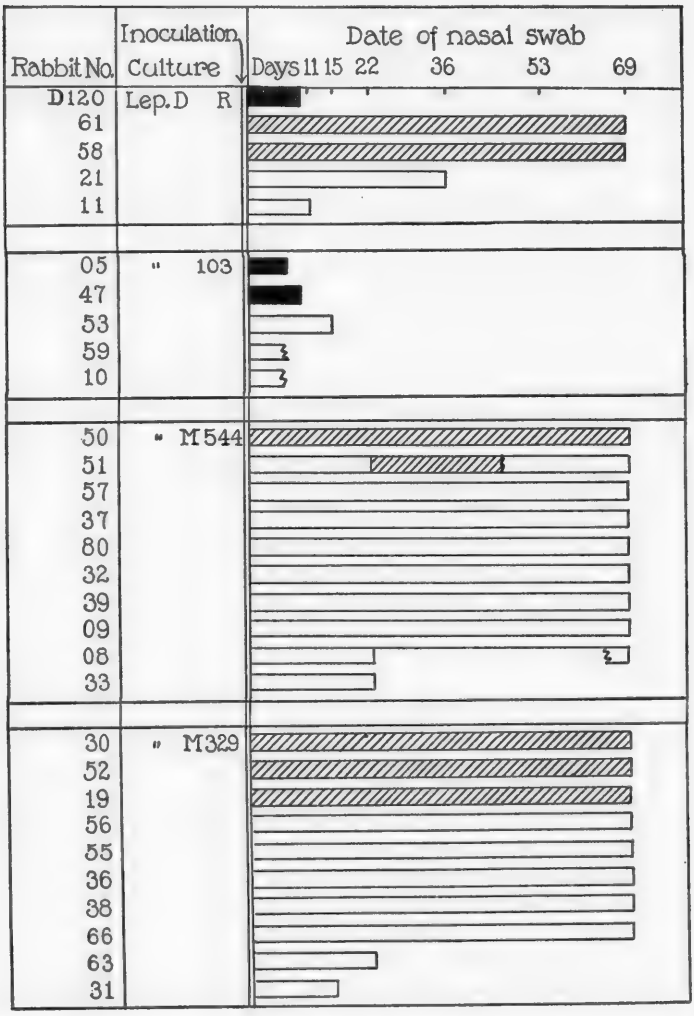
Rabbit No.	Date of inoculation May 9, 1924.					
	May 28, 1924.		June 4, 1924.		June 6, 1924.	
	D	G	D	G	D	G
D24	Infinity.	0	0	Infinity.	0	Infinity.
D18	+	+	++	++	++	++
D22	Infinity.	0	0	Infinity.	0	Infinity.

In the next experiment we compared the vegetative capacity of the D and mucoid strains of *Bact. lepisepticum.*

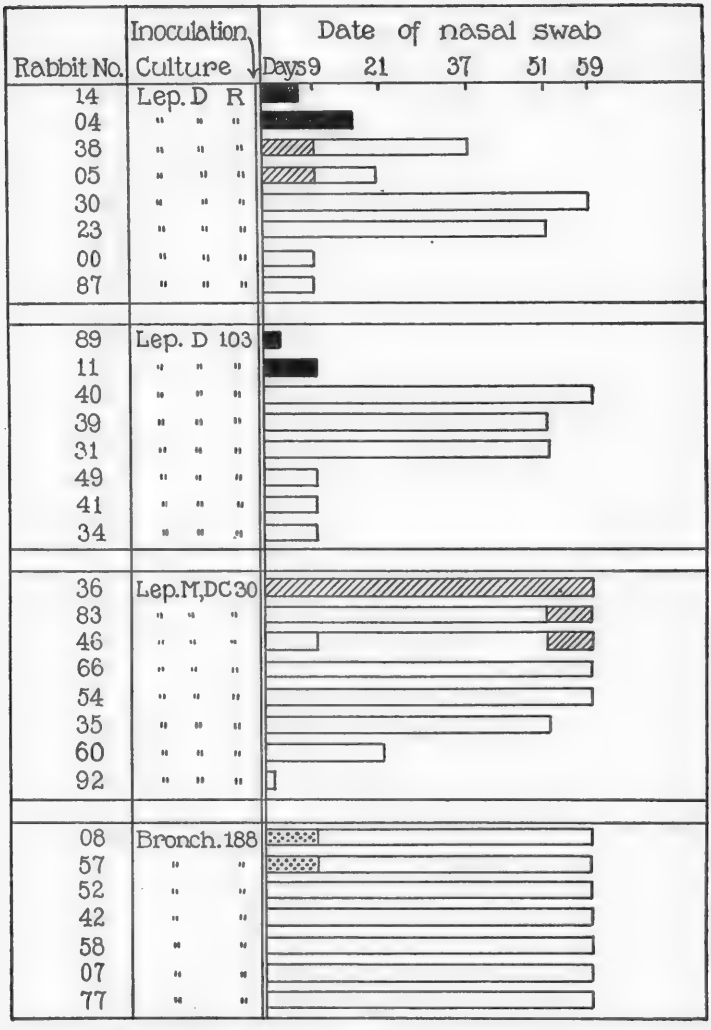
Experiment 2.—On February 17, 1925, thirty rabbits were given into each naris ¼ cc. (250,000,000 organisms) of various cultures of *Bact. lepisepticum.* Five received the Rivers D and five the No. 103 D strain; ten were given a mucoid strain, No. 544, and ten a mucoid strain, No. 329. Temperatures and nasal cultures were taken as before, and the survivors were autopsied and sectioned after a 73 day period of observation.

The results of these inoculations are pictured in Text-fig. 2. One rabbit, which received the Rivers D strain, died of pneumonia on the 9th day; two developed snuffles and were carriers throughout the period of observation; two showed no gross evidence of snuffles, but carried the organisms 11 and 36 days. Two of the No. 103 D series died of pneumonia on the 7th and 9th days; one carried the bacilli 15 days, and two less than 11 days. Two of the mucoid No. 544 series developed chronic snuffles; six others were "healthy" carriers throughout the experimental period. Four of the mucoid No. 329 series developed snuffles; five became "healthy" chronic carriers, while in one case the organisms were not recovered from the nares after the 22nd day.

A striking difference in the number of "healthy" carriers is again evident; eleven animals—55 per cent—of the mucoid, as compared with *none* of the D series. This result agrees with those of the previous experiment, and indicates that within the same, as well as in different bacterial species, there are some strains which are highly virulent and incapable of establishing a relatively harmless equilibrium with the host, whereas there are others of lower pathogenicity which



TEXT-FIG. 2. Response of specially bred rabbits to intranasal inoculation of *Bact. lepisepticum*, D and mucoid types. Experiment 2.



TEXT-FIG. 3. Response of specially bred rabbits to intranasal inoculation of *Bact. lepisepticum*, D and mucoid types, and *B. bronchisepticus*. Experiment 3.

do vegetate readily under both natural and experimental conditions.

In the next experiment, D and mucoid strains of *Bact. lepisepticum* and a strain of *B. bronchisepticus* were used.

Experiment 3.—On April 14, 1925, thirty-two rabbits received cultures as follows: Eight were given the Rivers D and eight the No. 103 D strain; eight a mucoid strain, No. DC 30, from the New City rabbit farm; and eight *B. bronchisepticus*, No. 188. Nasal cultures were taken at frequent intervals and clinical observations were recorded. After 62 days, the survivors, together with a control group of thirty normal rabbits, were killed and autopsied as in the previous experiments.

The results of the experiment are shown in Text-fig. 3, and may be stated as follows: Two of the eight rabbits receiving the Rivers D culture died of pneumonia; two showed symptoms of snuffles for a few days; one was a "healthy" carrier throughout the entire period of observation; one carried the organism for 51 days, and the other two for less than 9 days. From the nares of Rabbit 05 G variants were recovered which seemed to replace the original D strain and then disappear altogether. The animals receiving the 103 D culture behaved similarly. Two died of pneumonia; one became a chronic "healthy" carrier; two carried the organisms for 51 days, and the other three for less than 9 days. Three rabbits receiving the mucoid DC 30 culture developed snuffles; two became "healthy" carriers, and the other three harbored the bacteria for shorter periods of time. All of the rabbits given the *bronchisepticus* culture became chronic "healthy" carriers. Two showed a slight mucous discharge for 5 days.

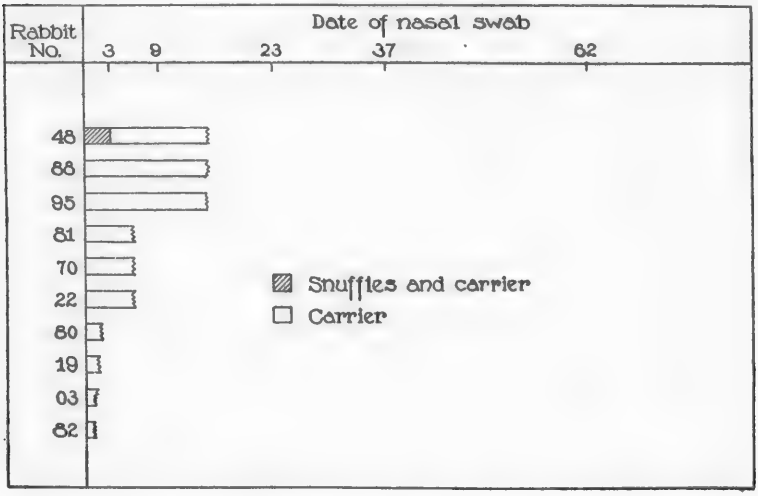
The low carrier rate in the D series—12.5 per cent—and the relatively high rates in the mucoid—25 per cent—and *B. bronchisepticus*—100 per cent—groups are consistent with the results obtained in the previous experiments, under similar conditions, and, what is even more important, correspond to conditions as they exist in nature at the present time—many "healthy" chronic carriers of *B. bronchisepticus* and the mucoid strains of *Bact. lepisepticum*, in contrast to very few carriers of D strains of *Bact. lepisepticum*. We conclude, therefore, that these *B. bronchisepticus* cultures and the mucoid strains of *Bact. lepisepticum*, both of low virulence, are more capable of a vegetative existence in the respiratory passages of rabbits than the highly virulent D strains.

One further test was carried out with a G variant culture.

Experiment 4.—On June 30, 1925, ten rabbits received in each naris ½ cc. of an 18 hour blood broth culture of the G variant of the Rivers D strain of *Bact. lepi-*

septicum. The animals were then placed under observation and cultured frequently. 64 days later, the survivors were killed and nasal tissues preserved for further study.

The results are plotted in Text-fig. 4. Four rabbits were free of the organisms within 3 days after inoculation, three others before the 9th day, and the remaining three before the 23rd day. One of the latter, No. 48, showed a serous discharge from the nares for 72 hours.



TEXT-FIG. 4. Response of specially bred rabbits to intranasal inoculation of *Bact. lepisepticum*, Type G. Experiment 4.

Evidently, therefore, pure cultures of the non-virulent G variant of the Rivers D strain do not vegetate easily in the nasal passages of normal rabbits.

Histological Studies of the Nasal Passages of Normal and Infected Rabbits.

It will be recalled that the nasal passages of each rabbit used in the preceding experiments were cut into three blocks and sectioned for histological study. The results of these examinations are described in the following paragraphs.

The forty-two control animals were obtained from the Rockefeller

Institute breeding room. Their nasal passages were free of *Bact. lepisepticum* and *B. bronchisepticus*, and contained only *Micrococcus catarrhalis* and other Gram-negative and positive cocci. At autopsy, heart's blood and lung cultures were negative. No gross abnormalities of any sort were observed except occasional pin worms in the intestines and *Encephalitozoon cuniculi* infection of the kidneys and brain.

Anterior, medial, and posterior sections through the nasal passages of twenty-seven of these animals appeared normal. The nasal septum was slender, the many folds of the turbinates delicate, and the air spaces free of mucus or cell débris. The areolar tissue surrounding the cartilage and blood vessels contained very few leucocytes. A single layer of cuboidal epithelium covered the surface of the septum and papillæ. Lymphoid tissue, either in definite nodules with germinal centers on the posterior wall, or as aggregations of lymphocytes scattered about on the septum or papillæ, was present in varying amounts (Figs. 1, *a* and 2, *a*). Occasionally, when an animal had been killed by an anesthetic or cervical blow, there was found a marked dilatation of the septal and papillary blood vessels and an abundance of fluid in the areolar tissue, nearly obliterating the free air spaces (Figs. 1, *b* and 2, *b*).

Very slight chronic inflammatory lesions of the septum or papillæ were present in fifteen cases. They consisted in local accumulations of eosinophils and mononuclear cells, hyperplasia of connective tissue, and a proliferation of the epithelial cells. Such changes as these made it appear that young, supposedly normal rabbits, kept under ideal conditions and free of the more dangerous respiratory pathogens, do, nevertheless, come into contact with subtle agents which induce in the tissues of the upper respiratory tract microscopic inflammatory changes. These lesions of the "controls" must, therefore, be kept in mind throughout the subsequent examination and interpretation of the experimental groups.

In general, the histological lesions in the nasal passages of the experimental animals differed in degree rather than in kind. In each experiment, the rabbits which resisted infection were like the controls in that they showed few or no abnormalities. Lymphoid tissue was relatively abundant. The "healthy" carriers usually showed focal chronic inflammatory lesions, which involved the septa or papillæ.

The epithelium was cuboidal and hyperplastic; sometimes it became columnar and exuberant with folds which gave the papillæ a cauliflower-like appearance (Figs. 1, *c* and 2, *c*). In these cases, eosinophils and mononuclears were numerous and proliferating fibroblasts increased the size and distorted the outlines of the septa and papillæ (Fig. 2, *d*). In cases with chronic snuffles, extreme examples of this process were encountered. The septum was thickened; papillæ were reduced in number and appeared large and blunt. Cauliflower-like proliferation of the columnar epithelium was striking. Fibroblasts and leucocytes distorted the cartilage and scattered the blood vessels. Lymphatic tissue was reduced in amount and cellular débris filled the lumina (Fig. 1, *d*). The nasal tissues of the animals which died of pneumonia were apt to be more nearly normal, except for generalized congestion, edema, and leucocytic accumulations, especially marked in the region of the lymphoid tissue.

The findings in the rabbits used in Experiment 1 may be summarized as follows: With the exception of the snuffles cases which showed erosion of the cartilage and reduction in number of papillæ, lesions in the *Bact. lepisepticum* group did not differ materially from those of the *B. bronchisepticus* series. Two of the four animals resistant to the Rivers strain were quite normal; the other two showed a single thickened area on the septum and one or two slightly enlarged papillæ. The two carrier cases had definite lesions in their septa, and slight hypertrophy of some papillæ. In seven of the eleven snuffles cases the septa were affected; in four the papillæ were reduced in number, very large and blunt, and covered with hyperplastic, columnar epithelium. Both of the pneumonia cases showed congestion, edema of the areolar tissue, and infiltrations of eosinophils. In the *B. bronchisepticus* group, eleven of the fourteen carriers had septal lesions and three cauliflower-like hyperplasia of columnar epithelium. In three cases nothing abnormal was noted.

The four different strains of *Bact. lepisepticum* used in Experiment 2 induced similar lesions of different severity. The nasal passages of the two animals resistant to the Rivers D strain appeared normal; those of the two snuffles cases showed marked thickening of the septa, a decrease in the number of papillæ, and proliferation of fibroblasts and columnar epithelium. One of the animals resistant to 103 D had slight septal lesions; the other two were normal. The one rabbit resistant to M 544 showed no nasal lesions. Of the five carriers examined, all had slight or moderate thickening of the septa and in three the papillæ were enlarged and the epithelium hyperplastic and columnar. One of the resistant animals in the M 329 series was normal; the other had a slight septal lesion. All of the five carrier cases showed marked chronic, inflammatory changes in the septum; in one

there was also an extensive involvement of the papillæ. The three snuffles cases had severe lesions. Fibroblasts and proliferating columnar epithelium thickened the septum and distorted the papillæ. Mononuclear cells were scattered about in large numbers.

The same types of acute and chronic inflammatory processes were found in the nasal passages of rabbits used in Experiment 3. The two resistant animals in the *Bact. lepisepticum* Rivers D group had slight lesions on their septa, with thickened nodules of columnar epithelium on the papillæ. The one carrier showed slight thickening of the septum and one or two club-shaped papillæ. Two rabbits which had had symptoms of snuffles for a short time only, and had ceased to carry the organisms, had marked areas of thickening on the septa and slight hyperplasia of the papillary epithelium. One of the two pneumonia cases showed a slight nodule on the septum; the papillæ of both were edematous, congested, and infiltrated with eosinophilic leucocytes. There were three resistant animals in the *Bact. leposepticum* D 103 series. Two were normal, and one had a chronic, inflammatory nodule on the septum. The three carriers for part or the whole of the period had marked thickening of the septa and enlarged papillæ covered with hyperplastic, columnar epithelium. The one pneumonia case examined showed the typical, acute, inflammatory reaction. The one animal resistant to *Bact. lepisepticum* DC 30 had a slight septal lesion. The carriers and those with snuffles showed marked thickening of the septa and cauliflower-like enlargement of the papillæ, due to hyperplasia of columnar epithelium. All the carriers in the *B. bronchisepticus* group had connective tissue thickening and epithelial hyperplasia of the septa. Three showed, besides, extreme cauliflower-like proliferation of columnar epithelium on the papillæ.

Four of the rabbits inoculated with the Rivers G strain showed no lesions in their nasal passages. Four had slight lesions of the septum; two of these and two others had slight foci on one or two papillæ.

These findings are summarized in Table II in such a way as to show the presence or absence of lesions in the nasal passages of (1) control, uninoculated rabbits; (2) similar animals which proved refractory to nasal instillations of *Bact. lepisepticum* or *B. bronchisepticus;* (3) animals which became chronic "healthy" carriers of these organisms; and (4) those which developed snuffles, or (5) pneumonia. The totals at the foot of the table state that 65 per cent of the controls showed no lesions in their nasal passages; that a relatively similar number—56 per cent—of the refractory animals was normal; that 92 per cent of the healthy, chronic carriers had lesions; and that all of the snuffles and pneumonia cases showed extensive chronic or acute, inflammatory reactions. From these figures we conclude that the animal which is

TABLE II.

Histological Findings in the Nasal Passages of Resistant Rabbits, "Healthy" Carriers, Snuffles, and Pneumonia Rabbits (Bact. lepisepticum and B. bronchisepticus).

Experimental series.	Clinical and bacteriological results.																				
	Control group.					"Resistant" group.					"Healthy" carrier group.					Snuffles group.			Pneumonia group.		
	Histological findings.					Histological findings.					Histological findings.					Histological findings.			Histological findings.		
	Total No.	Normal.		Slight lesions.		Total No.	Normal.		Slight focal lesions.		Total No.	Normal.		Moderate, chronic inflammatory lesions.		Total No.	Chronic inflammatory lesions.		Total No.	Acute inflammatory lesions.	
		No.	%	No.	%		No.	%	No.	%		No.	%	No.	%		No.	%		No.	%
Controls	42	27	65	15	35																
Experiment 1.																					
Lep. Rivers D.	—	—	—	—	—	4	2	50	2	50	2	0	—	2	100	11	11	100	2	2	100
Bronch.	—	—	—	—	—	0	—	—	—	—	14	3	21	11	79	0	—	—	0	—	—
Experiment 2.																					
Lep. Rivers D.	—	—	—	—	—	2	2	100	—	—	0	—	—	—	—	2	2	100	1	1	100
Lep. 103 D	—	—	—	—	—	3	2	66	1	33	0	—	—	—	—	0	—	—	2	2	100
Lep. M 544	—	—	—	—	—	1	1	100	—	—	7	—	—	7	100	2	2	100	0	—	—
Lep. M 329	—	—	—	—	—	2	1	50	1	50	5	—	—	5	100	3	3	100	0	—	—
Experiment 3.																					
Lep. Rivers D.	—	—	—	—	—	2	0	—	2	100	1	0	0	1	100	2	2	100	2	2	100
Lep. 103 D	—	—	—	—	—	3	2	66	1	33	1	0	0	1	100	0	—	—	2	2	100
Lep. M DC 30	—	—	—	—	—	1	0	0	1	100	2	0	0	2	100	3	3	100	0	—	—
Bronch. 188	—	—	—	—	—	0	—	—	—	—	7	0	0	7	100						
Experiment 4.																					
Lep. Rivers G.	—	—	—	—	—	7	4	57	3	43	0	—	—	—	—	0	—	—	0	—	—

	Controls.		Resistants.		Carriers.		Snuffles.	Pneumonia.
	% normal.	% slight lesions.	% normal.	% slight lesions.	% normal.	% lesions.	% chronic inflammatory lesions.	% acute inflammatory lesions.
Summary of totals	65	35	56	44	8	92	100	100

refractory to a nasal instillation of these organisms is actually not infected, and that the chronic carrier, although clinically healthy, is, in truth, an infected, although relatively resistant, individual.

The fact that the lesions found in the controls seemed to differ in degree only from those found in animals infected experimentally with *Bact. lepisepticum* and *B. bronchisepticus* might lead one to consider that some more subtle agent was responsible for the entire process, and that the introduced bacteria served only to aggravate a condition already present. However, all other clinical, bacteriological, and pathological evidence makes this interpretation most unlikely. Rather one must regard the nasal passages as delicate tissues which respond in a characteristic manner to various types of injurious influences.

DISCUSSION.

Fundamental questions regarding the significance of the "healthy" chronic carrier state have been coming more and more into the foreground. These questions relate to possible changes in the bacteria, as well as to modifications in the host. For example, it has been noted that the diphtheria bacilli[2] and the pneumococci[3] which survive in the nasopharynx usually belong to the less virulent groups. Mucoid and rough variants of typhoid, paratyphoid, and dysentery bacilli have also been recovered from the stools of human carriers.[4]

In the study of these bacterial types and variants from human carriers, it has been necessary to base conclusions regarding virulence and vegetative properties upon the results of artificial animal tests.[5] It may, therefore, be considered as an advantage to study these

[2] Guthrie, C. G., Gelien, J., and Moss, W. L., *Bull. Johns Hopkins Hosp.*, 1920, xxxi, 388.

[3] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1915, xxii, 105. Christensen, S., Studies on pneumococcus types, Thesis, Copenhagen, 1923, in *Communications de l'Institut sérothérapie de l'Etat Danois*, 1923, xiv, No. 5.

[4] Fletcher, W., *Lancet*, 1918, ii, 102; *J. Roy. Army Med. Corps*, 1920, xxxiv, 219. Krumwiede, C., Cooper, G., and Provost, D. J., *J. Immunol.*, 1925, x, 55. Gardner, A. D., and Walker, E. W. A., *J. Hyg.*, 1921, xx, 110. Walker, E. W. A., *Proc. Roy. Soc. London, Series B*, 1922, xciii, 54; *J. Hyg.*, 1922–23, xxi, 87.

[5] Schoenholz, P., and Meyer, K. F., *J. Infect. Dis.*, 1921, xxviii, 588. Christiansen, C. R., Neilson, N. M., and Meyer, K. F., *J. Infect. Dis.*, 1921, xxviii, 394.

properties in a bacterium which induces respiratory infection in rabbits under natural conditions, which conditions may be more closely simulated. Our own experiments, reported in this paper, have shown decisively that highly virulent types of *Bact. lepisepticum* are incapable of surviving in the nasal passages of the healthy rabbit, while strains of low virulence, including several cultures of *B. bronchisepticus*, both survive and multiply for long periods. We found also that in the more resistant animals, the highly virulent strains of *Bact. lepisepticum* are replaced by variants of very low pathogenicity, which themselves disappear altogether after a short time.

Nor is this all. For we learned that the equilibrium of the carrier state implies a selection of certain individuals as hosts and a definite reaction in their tissues. If any considerable number of healthy, non-exposed rabbits are given virulent cultures by nasal instillation, the "healthy" chronic carrier condition develops only in individuals relatively resistant to the organism, and induces always, in these animals, a definite, chronic, inflammatory process. These observations are in agreement with those of "healthy" chronic, human carriers, who are now regarded as infected individuals displaying a certain amount of immunity.[6] Hence, the two series of observations—the one derived from human, and the other from experimental animal pathology—are in this respect in agreement.

As a conclusion to this report, we wish to summarize the results of all our studies of host susceptibility in *Bact. lepisepticum* infection, and to relate them to those already obtained in our investigation of mouse typhoid. In the first place, we have observed that the rabbits in each of several communities, in which a uniform type of *Bact. lepisepticum* is widespread, may be divided into the following, definite groups, according to their reaction to the presence of this organism: (1) non-infected, resistant animals; (2) chronic "healthy" carriers; (3) cases with local infections, such as snuffles and otitis media; and

[6] Ledingham, J. C. G., and Arkwright, J. A., The carrier problem in infectious diseases, International Medical Monographs, London, 1912. Garbat, A. L., Typhoid carriers and typhoid immunity, Monograph of The Rockefeller Institute for Medical Research, No. 16, New York, 1922. Nichols, H. J., and Kelser, R. A., Carriers in infectious diseases, Baltimore, 1922. Dudley, S. F., *Pub. Health*, 1922–23, xxxvi, 61.

(4) pneumonia and septicemia cases.[7, a, b, c] Secondly, we are finding that pure bred races of rabbits differ markedly in their susceptibility to this organism.[7, d] We note, furthermore, that other influences—seasonal changes[7, a] and harmful substances injected parenterally[7, e]—disturb this equilibrium between parasite and host, and lead to an increase in percentage of snuffles and pneumonia cases and a corresponding decrease in chronic carriers and resistant animals.

Fortunately, we have been able to induce these various types of reaction under experimentally controlled conditions, with known and constant bacterial virulence and dosage and specially bred rabbits, protected from previous exposure to *Bact. lepisepticum.* Under these circumstances, if any group of such rabbits is given a known dose of a culture of *Bact. lepisepticum*, by nasal instillation, the percentage of resistant animals, of chronic carriers, and of snuffles and pneumonia cases is relatively constant and may be considered to express quantitatively the resistance of the given group of animals to the organism ([7, c, f] and this communication).

It would require many hundreds of rabbits to convert these rough percentages into a curve which would describe exactly the resistance of an inbred strain of rabbits to a given dose of any type of *Bact. lepisepticum.* But all the data thus far accumulated indicate that rabbits show the same highly various susceptibility to *Bact. lepisepticum* as mice do to mouse typhoid bacilli, and that for rabbits, as well as for mice, "this quality of resistance is present in different amounts in individuals of the same family or race, and that these differences, under properly controlled conditions, take the form of a frequency curve."[7, h]

SUMMARY.

Rabbits with no previous exposure to *Bact. lepisepticum* were given, intranasally, a known dose of one strain or another of this organism, or of *B. bronchisepticus.* The results give grounds for the following conclusions.

[7] Webster, L. T., (*a*) *J. Exp. Med.*, 1924, xxxix, 837; (*b*) xl, 109. (*c*) Smith, D. T., and Webster, L. T., *J. Exp. Med.*, 1925, xli, 275. (*d*) Webster, L. T., *Am. J. Hyg.*, 1925, v, 335; (*e*) *J. Exp. Med.*, 1924, xxxix, 857; (*f*) xl, 117; (*g*) 1926, xliii, 555; (*h*) 1925, xlii, 1.

(*a*) Results referable to the fate of the bacteria.

1. Highly virulent strains of *Bact. lepisepticum* are incapable of vegetating indefinitely in the nasal passages of surviving rabbits. But from the organism of high virulence, variants of low pathogenicity may develop and persist for a few days.

2. Less virulent, mucoid strains of *Bact. lepisepticum* and strains of *B. bronchisepticus* are capable of surviving indefinitely in the rabbit's nasal passages.

(*b*) Results referable to the host.

1. Rabbits refractory to infection under the conditions of the experiment usually show no lesions of the nasal passages, other than those to be met with in control rabbits. These may show slight microscopic, focal, chronic, inflammatory lesions of the nasal passages.

2. The animals which become chronic "healthy" carriers, as result of the infection, and those which show clinical symptoms of snuffles all develop well marked, chronic, inflammatory lesions of the nasal passages.

3. Those markedly susceptible animals which succumb to pneumonia caused by the injected organism all show an acute type of inflammatory lesion of the nasal passages.

EXPLANATION OF PLATES.

PLATE 24.

FIG. 1, *a* to *d*. Nasal passages of control and experimental rabbits. × 5.

(*a*) Normal control rabbit. Note delicate septum and papillæ.

(*b*) Normal control rabbit killed with chloroform. The blood vessels and lymph and tissue spaces are so engorged that the breathing space is greatly reduced.

(*c*) Control Rabbit D318. There are local areas of thickening on the septum and a number of papillæ with club-shaped ends.

(*d*) Experimental Rabbit D236. Inoculated intranasally with *Bact. lepisepticum* DC 30. Clinical snuffles was apparent during the 2 months period of observation. Note the reduction in number and increase in size of the papillæ on the left side. There is a small abscess involving one turbinate.

PLATE 25.

FIG. 2, *a* to *d*. Sections of papillæ and septum of the nasal cavities of normal and experimental rabbits. × 135.

(*a*) Normal control rabbit. A single layer of cuboidal epithelium covers the cartilage and delicate areolar tissue.

(*b*) Normal control rabbit killed with chloroform. The blood and lymph vessels are distended and the tissue spaces are filled with serum.

(*c*) Experimental Rabbit D331. Inoculated intranasally with *Bact. lepisepticum* 103. Proliferating fibroblasts and hyperplasia of columnar epithelium have given the papillæ a cauliflower-like appearance.

(*d*) Nasal septum of the same rabbit. Note the fibroblasts and leucocytes beneath the hyperplastic, exuberant, columnar epithelium.

FIG. 1.

(Webster: Respiratory infections in rabbits. VIII.)

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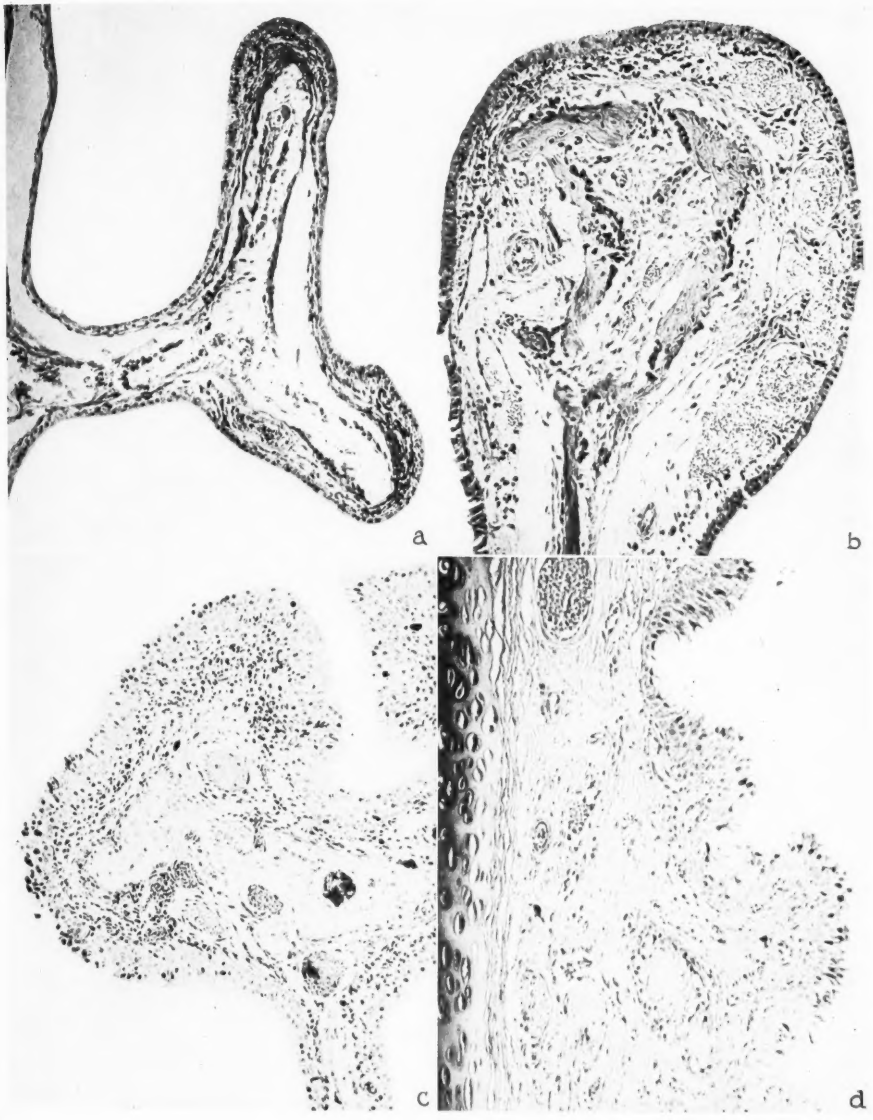

FIG. 2.

(Webster: Respiratory infections in rabbits. VIII.)

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